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THE EFFECTS OF EPINEPHRINE ON THE METABOLISM OF 17-HYDROXYCORTICOSTEROIDS IN THE HUMAN*

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INTRODUCTION

THE factors regulating the secretion of the adrenocorticotropic hormone (ACTH) by the adenohypophysis and consequently those governing the secretion of adrenal corticosteroids have not been well defined. Cannon demonstrated some years ago that many stressful situations are accompanied by the release of epinephrine from the adrenal medulla and by stimulation of the sympathetic nervous system (1, 2). Subsequently Selye and others presented evidence that the effects which

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are produced by adrenocortical hormones are accentuated after stress—the "alarm reaction" of Selye (3–6). Because somewhat similar effects may be produced by epinephrine administration, it has been postulated that epinephrine may be the so-called "common-denominator" in stimulating the pituitary-adrenal system under conditions of stress (7, 8).

Although many investigators have agreed that epinephrine is capable of inducing release of ACTH by the anterior pituitary, the evidence has been based in most instances on the development of changes resembling adrenocortical effects; direct measurements of ACTH or adrenocortical hormones have not been available. In addition, no agreement has existed as to the manner in which such stimulation was induced. Long and coworkers have been proponents of the theory that epinephrine specifically and directly stimulates the anterior pituitary gland to secrete ACTH (9–12). Sayers and Sayers (4, 5, 13), on the other hand, have suggested that epinephrine increases the peripheral utilization of adrenal corticosteroids and that the resulting decreased level of these steroids stimulates the adenohypophysis to release ACTH. Some evidence has accumulated in the meantime which suggests the possibility of direct stimulation of the adrenal cortex by epinephrine (14, 15). Still other workers have regarded the hypothalamic region as the main relay center for stimuli to the anterior pituitary gland (16–19), epinephrine merely being one of the many factors capable of activating the center.

The injection of epinephrine has been used in man and in animals as a test to determine whether or not the pituitary-adrenal system is intact, the drop in circulating eosinophils being used as the most easily determinable and sensitive indicator (20–23). However, several findings have suggested that the action of epinephrine on the pituitary-adrenal system is complicated and bizarre. Frequent and prolonged administration of epinephrine does not result in a significant increase in the urinary excretion of adrenal corticosteroids or of 17-ketosteroids (20, 24); nor does it relieve the symptoms of rheumatoid arthritis (24). The electrolyte changes which follow epinephrine administration do not resemble those which occur after the administration of either ACTH or cortisone (25–27). Significant decreases in the circulating eosinophils can be produced with epinephrine in adrenalectomized and in hypophysectomized animals, as well as in certain patients with Addison's disease and in adrenalectomized patients receiving small doses of cortisone (20, 22, 28–31). These changes are more pronounced when additional 17-hydroxycorticosteroids are also administered (30).

In this paper the effects of epinephrine on the peripheral blood levels of 17-hydroxycorticosteroids in humans are described.

It has been demonstrated that the administration of ACTH will increase

the 17-hydroxycorticosteroid level of the blood as well as the output of these steroids by the adrenal gland (32, 33). If epinephrine causes production of ACTH by the direct stimulation of the anterior hypophysis or indirectly by stimulation of the hypothalamus, an increase in blood 17-hydroxycorticosteroid levels should follow. Therefore, levels of the 17-hydroxycorticosteroids in the peripheral circulation were measured after intravenous and subcutaneous administration of epinephrine. Epinephrine was given in various doses and over varying periods of time. In a few cases the urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids was also measured. The changes in eosinophils, lymphocytes and polymorphonuclear cells, as well as in the total leukocyte count, were determined simultaneously.

METHODS AND MATERIALS

Most of the observations were made on normal young males who had become well acquainted with the experimental procedures and had served as subjects on other occasions. Before the infusion of epinephrine was begun all subjects had normal pulse rates and blood pressures and no apprehension of the approaching procedure was exhibited. All subjects were in the fasting state and all experiments were begun between 8:00 and 9:00 A.M. The pulse rate and blood pressure were checked every two to five minutes. In all instances subjective symptoms, tachycardia, elevation of systolic blood pressure and a decrease in diastolic blood pressure developed and persisted throughout the period of infusion. Blood samples (usually 30 ml.) for determination of steroid and cell content, were drawn immediately before the experiment and at varying intervals thereafter. Control experiments on the same subjects were performed following injection of steroids and ACTH under nearly identical conditions. The same lot of epinephrine was used in each series of experiments.

Several observations were made on a subject with Addison's disease. Except for the somewhat smaller doses of epinephrine given the patient, the same procedures were carried out. Several months prior to the experiments, pellets of desoxycorticosterone acetate had been implanted in the patient and he had not received cortisone or any other 11,17-oxysteroid for two weeks before the experiments. He had previously undergone several Addisonian crises, but for six months before the experiment he had felt well and had been carrying on his usual activities.

Epinephrine (1:1,000 solution; Parke, Davis and Company or Metropolitan Laboratories, Inc.) was given in 250 ml. of saline at a constant rate. That no inactivation of the epinephrine occurred is witnessed by the fact that slight speeding up of the rate of infusion even after four hours resulted in prompt, unpleasant and occasionally alarming subjective

symptoms, a further increase in tachycardia and a change in blood pressure. Compound E (17-hydroxy-11-dehydrocorticosterone) and compound F (17-hydroxy-corticosterone) were given intravenously in amounts varying from 25 to 100 mg., as the free alcohols in normal saline solution containing 5 to 10 per cent ethanol. The same volume of fluid was used on each occasion and the conditions of infusion were identical. ACTH was dissolved in saline. Urine was collected without preservative, immediately refrigerated and analyzed within forty-eight hours.

The methods for hematologic estimations, for the determination of 17-hydroxycorticosteroids in blood and urine, and for urinary 17-ketosteroids have been described previously (34-36). In this paper the term 17-hydroxycorticosteroids (17-OH corticosteroids) is used to refer to adrenal cortical steroids with a 17,21-dihydroxy-20 ketone group, such as Kendall's compounds E and F and Reichstein's substance S. The 17-ketosteroids and 17-hydroxycorticosteroids measured in urine are those released by β-glucuronidase hydrolysis. By these methods, average normal values of urinary excretion for males are approximately 4.5 mg. of 17-hydroxycorticosteroids and 6.9 mg. of 17-ketosteroids per twenty-four hours. The upper limits of 17-OH corticosteroid concentration in the blood of normal humans is approximately 23 μg./100 ml. of plasma (12 μg./100 ml. of whole blood[1]) by the technique referred to.

RESULTS

Effects of administration of epinephrine on 17-hydroxycorticosteroid levels in the human

Intravenous epinephrine.—The results of the administration of 1.0 mg. of epinephrine intravenously over four hours to Subject C.D. are shown in Table 1. The significant finding is the lack of rise in 17-hydroxycorticosteroid levels in the blood during or after the infusion, in contrast to the rather profound changes in the polymorphonuclear neutrophils (PMN's) and eosinophils. The changes in 17-OH corticosteroid levels were of no greater magnitude than the usual diurnal variation.

On the assumption that 1.0 mg. of epinephrine may have been too small a dose to stimulate the pituitary-adrenal system, Subjects A.D. and A.L. were given 2.5 mg. and 3.0 mg. of epinephrine, respectively, over a period of four hours. Furthermore, because of the possibility that an increase in the amount of 17-OH corticosteroids may have occurred in Subject C.D. before the one hour sampling, 17-OH corticosteroids in the blood were determined on Subject A.L. at fifteen, thirty and forty-five minutes after

[1] These steroids are present in plasma but not in red blood cells. Therefore, values for whole blood are about one half as great per volume as values for plasma (33, 34).

TABLE 1. EFFECTS IN NORMAL MALES OF ADMINISTRATION OF EPINEPHRINE INTRAVENOUSLY (OVER A PERIOD OF FOUR HOURS) ON BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS AND ON LEUKOCYTES

Subject	Dose of epinephrine	Time after start of infusion	17-OH steroids (γ/100 ml. of plasma)	Leukocytes (absolute number per cu. mm.)			
				WBC	PMN	Lymph.	Eos.
C.D.	1 mg.	0 hr.	10	5,400	2,700	2,020	247
		1 hr.	11	4,475	2,202	1,781	138
		2 hr.	7	4,600	2,916	1,288	120
		4 hr.	9	5,800	4,073	1,265	47
		6 hr.	13	7,520	5,429	1,624	131
		8 hr.	13	6,275	4,128	1,657	119
A.L.	3 mg.	0 hr.	4	10,325	7,186	2,561	200
		15 min.	4	12,925	8,763	3,257	282
		30 min.	4	13,700	9,398	3,508	328
		45 min.	4	11,925	8,157	2,958	335
		1 hr.	<2*	12,900	8,462	3,277	228
		2 hr.	<2	16,350	12,818	2,747	119
		3 hr.	4	22,175	17,651	3,371	41
		4 hr.	8	18,100	14,770	2,643	28
		6 hr.	4	14,300	11,897	1,888	25
		8 hr.	19	14,675	11,828	2,201	156
A.D.	2.5 mg.	0 hr.	11	9,975	7,342	2,195	100
		1 hr.	9	12,525	8,567	3,282	206
		2 hr.	11	11,280	8,257	2,340	150
		3 hr.	17	13,300	9,363	3,298	112
		4 hr.	<2	16,075	11,831	3,312	94
		6 hr.	<2	11,500	9,269	1,771	44

* Because there is some doubt concerning the quantitative accuracy of the method at levels below 2 micrograms per 100 ml., all values below this level have been recorded as <2. Most such samples contained no detectable steroid.

the start of the infusion. Again, no rise in 17-OH corticosteroid levels was observed; in fact, no determinable levels were found at one and two hours. It should be pointed out, however, that neither the subsequent rise nor the drop in levels of 17-OH corticosteroids were outside the limits of normal variation in steroid levels (*see* subsequent section). In contrast, the cellular changes were marked; increases took place in the total white blood cell count and in the polymorphonuclear neutrophils and there was a significant decrease in eosinophils. The data for these experiments are shown in Table 1.

Since the foregoing doses of epinephrine may be considered "unphysiologic," smaller doses of epinephrine were administered by the intravenous

TABLE 2. EFFECTS OF SMALL INTRAVENOUS DOSES OF EPINEPHRINE (GIVEN OVER THE FIRST HOUR) ON THE BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS AND LEUKOCYTES IN NORMAL MALES

Subject	Dose of epinephrine	Time after start of infusion	17-OH steroids (γ/100 ml. of plasma)	Leukocytes (absolute number per cu. mm.)			
				WBC	PMN	Lymph.	Eos.
A.L.	0.4 mg.	0 hr.	16	7,425	4,544	2,183	184
		½ hr.	12	10,425	5,797	3,544	256
		1 hr.	9	7,800	4,368	2,590	125
		2 hr.	10	7,650	4,835	1,989	158
		3 hr.	12	10,925	7,648	2,491	100
		4 hr.	14	10,575	7,424	2,221	103
C.F.	0.25 mg.	0 hr.	20	7,400	3,271	3,049	366
		2 min.	17	8,125	2,925	4,079	544
		5 min.	17	9,850	3,487	5,063	748
		15 min.	20	9,675	3,367	4,625	574
		30 min.	14	9,375	3,994	4,125	862
		1 hr.	9	8,650	4,221	3,218	550
		2 hr.	10	9,060	5,979	2,102	306
		3 hr.	8	14,350	10,045	3,100	262
		4 hr.	8	12,000	7,368	3,192	294
		6 hr.	8	11,200	6,294	4,010	347
J.E.	0.1 mg.	0 hr.	8	5,050	2,687	1,677	182
		2 min.	<2	6,400	3,405	2,138	234
		5 min.	5	6,200	3,199	2,430	247
		15 min.	9	7,425	3,356	3,445	250
		30 min.	4	6,950	3,128	3,072	269
		1 hr.	6	6,525	2,727	3,028	238
		2 hr.	8	5,550	3,141	1,865	172
		3 hr.	10	7,825	4,758	2,160	138
		4 hr.	9	8,225	5,313	2,188	184
		6 hr.	9	7,550	4,167	2,416	268
		8 hr.	5	9,175	4,973	3,211	225

route. The results of these experiments are presented in Table 2. Subject A.L., who received 0.4 mg. of epinephrine over one hour, showed no significant changes in 17-OH corticosteroid levels and only a modest cellular response. The urinary excretion of 17-OH corticosteroids on a control day was 4.5 mg. and the 17-ketosteroid excretion was 5.0 mg. per twenty-four hours. On the day of epinephrine administration the 17-OH corticosteroid output in the urine was 4.3 mg. and the 17-ketosteroid output was 4.3 mg. per twenty-four hours. Subject C.F., who received 0.25 mg. of epinephrine intravenously over one hour, also showed no significant changes in 17-OH corticosteroids but there was a good cellular response. The drop in 17-OH

corticosteroid concentration during this six-hour period is not unusual during this period of the day (37). In Subject J.E., who received 0.1 mg. of epinephrine intravenously over one hour and showed very few of the cardiovascular and subjective effects of epinephrine, there was no significant change in 17-OH corticosteroids and the cellular response was poor.

Subcutaneous epinephrine.—The effects of subcutaneous administration of epinephrine were also studied because of the possibility that the prolonged and less intense stimulation produced by subcutaneous administration might lead to an increase in 17-OH corticosteroid levels in the blood. The results of such experiments are shown in Table 3. Subject J.B. received 0.3 mg. of epinephrine subcutaneously. No rise in steroids occurred; in fact, there was a fall in steroid levels at thirty minutes and one hour. The cellular changes, especially in polymorphonuclear neutrophils, were of good magnitude. On another occasion this subject, J.B., received a number of subcutaneous injections of epinephrine over a period of two days. On the first day he received 0.5 mg. of epinephrine subcutaneously at

TABLE 3. EFFECTS OF SUBCUTANEOUS DOSES OF EPINEPHRINE ON THE BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS AND LEUKOCYTES IN NORMAL MALES

Subject	Dose of epinephrine	Time after initial injection	17-OH steroids (γ/100 ml. of plasma)	Leukocytes (absolute number per cu. mm.)			
				WBC	PMN	Lymph.	Eos.
C.F.	0.5 mg.	0 hr.	20	6,225	2,042	3,449	429
	0.3 mg.	4 hr.	14	11,775	8,949	2,190	62
	0.3 mg.	8 hr.	<2	7,200	3,557	3,024	150
J.B.	0.3 mg.	0 hr.	22	4,825	2,036	2,258	116
		2 min.	21	4,750	1,815	2,375	99
		5 min.	18	4,700	1,899	2,331	147
		15 min.	15	5,925	1,884	3,543	106
		30 min.	6	6,625	2,730	3,299	106
		1 hr.	7	6,925	2,881	3,601	112
		2 hr.	20	6,750	3,632	2,524	75
		3 hr.	21	9,775	6,901	1,818	66
		4 hr.	18	11,900	8,306	2,737	72
		6 hr.	12	9,700	6,247	3,007	82
J.B.	0.5 mg.	0 hr.	21	6,625	2,902	3,061	162
	0.3 mg.	4 hr.	14	11,200	8,803	1,770	41
	0.3 mg.	8 hr.	15	9,850	6,127	2,896	88
	0.3 mg.	24 hr.	23	4,800	2,198	2,102	53
	0.3 mg.	28 hr.	11	6,700	3,377	2,559	134
	0.3 mg.	32 hr.	16	7,150	3,847	2,646	81
		48 hr.	21	4,875	2,301	2,018	116

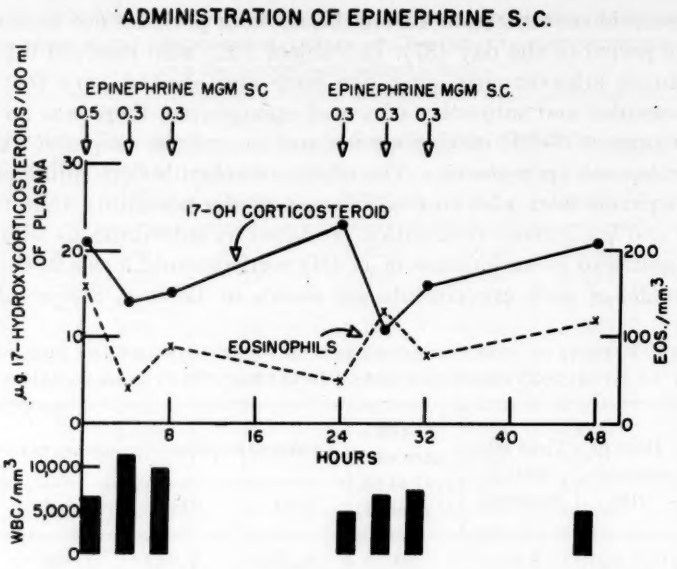

FIGURE 1

8:00 A.M., and 0.3 mg. at noon and 4:00 P.M. On the second day he was given 0.3 mg. of epinephrine at 8:00 A.M., and the same dose at noon and 4:00 P.M. The results are presented in Figure 1. Although the cellular changes were significant, no significant change in 17-OH corticosteroid blood levels occurred. The urinary 17-OH corticosteroids and 17-ketosteroids did not increase significantly. It is to be noted that the cellular changes were less intense on the second day.

Subject C.F. received a total of 1.1 mg. of epinephrine subcutaneously at intervals during the day, 0.5 mg. at 8:00 A.M., and 0.3 mg. at noon and 4:00 P.M. Although the cellular changes were marked, the 17-OH corticosteroid levels did not rise and actually no steroid was demonstrated at eight hours. The urinary excretion of 17-OH corticosteroids and 17-ketosteroids did not increase over the control twenty-four hours. As a matter of fact, the 17-OH corticosteroid excretion fell from an average control value of 5.3 mg. to 3 mg. per twenty-four hours on the day of epinephrine administration, while the 17-ketosteroids remained unchanged. Another subject, who received epinephrine in the same manner as C.F., showed an insignificant rise of 17-OH corticosteroid excretion in the urine; the excretion on control days averaged 6.5 mg., whereas it was 7.2 per twenty-four hours under treatment. On the other hand, the 17-ketosteroids decreased from a daily output of 4.5 mg. on control days to 2.0 mg. per twenty-four hours on the day of epinephrine administration.

TABLE 4. BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS AND LEUKOCYTES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF ADRENAL STEROIDS, WITH AND WITHOUT EPINEPHRINE, TO NORMAL MALES

Subject	Doses of steroids & epinephrine given, & length of time administered	Hours after start of infusion	*Adrenal steroids without epinephrine*					*Adrenal steroids with epinephrine*				
			17-OH steroids (γ/100 ml. of plasma)	Leukocytes (absolute number per cu. mm.)				17-OH steroids (γ/100 ml. of plasma)	Leukocytes (absolute number per cu. mm.)			
				WBC	PMN	Lymph.	Eos.		WBC	PMN	Lymph.	Eos.
A.L.	Cmpd. E (free alcohol), 100 mg.	0	8	6,375	3,965	1,938	163	8	6,125	4,042	1,617	131
	Epinephrine, 2 mg.	1	42	6,025	3,711	1,844	116	49	12,125	6,814	4,389	147
	Both given over 4 hrs.	2	44	7,025	5,030	1,602	50	62	17,125	13,529	2,706	59
		3	47	7,950	6,535	1,145	38	75	22,800	20,155	1,824	21
		4	74	9,075	7,877	998	40	95	19,275	18,234	810	0
		6	25	10,825	9,699	844	19	32	12,575	11,846	629	3
		8	12	9,975	8,778	858	28	20	12,625	11,085	1,364	0
C.C.	Cmpd. E (free alcohol), 100 mg. over 1 hr.	0	19	8,400	5,595	2,150	294	19	7,825	4,022	2,986	357
		1	74	8,550	5,917	2,172	250	150	13,150	6,444	5,654	400
		2	55	8,900	6,408	1,976	156	60	14,100	10,096	3,243	138
	Epinephrine, 3 mg. over 4 hrs.	3	25	9,300	7,236	1,562	144	39	17,100	13,543	2,770	38
		4	27	9,400	7,952	1,242	31	33	20,925	17,870	2,385	6
		5	24	11,075	8,949	1,883	6	24	11,725	10,506	938	3
		7	13	11,625	8,905	2,348	53	17	15,500	12,927	1,767	3
		9	3	13,425	9,935	2,980	125	22	17,750	13,561	3,337	34
		24	2	12,750	9,154	2,933	172	20	9,175	5,358	3,156	166
C.F.	Cmpd. F (free alcohol), 25 mg. over ½ hr.	0	10	7,775	3,966	2,816	577	4	6,050	2,517	2,722	400
		½	64	7,850	3,925	3,077	357	75	9,000	3,456	4,392	412
		1	31	7,900	4,250	3,049	338	38	10,050	4,120	4,864	512
	Epinephrine, 1.5 mg. over 3 hrs.	2	24	8,000	5,264	2,240	288	34	14,120	10,392	2,881	128
		3	15	7,375	5,074	1,903	182	16	20,450	17,669	2,045	22
		4	15	8,475	5,407	2,661	210	20	16,850	14,390	1,887	16
		6	8	8,000	4,400	2,832	235	20	14,120	10,844	2,599	6
		8	5	8,775	4,054	3,843	288	12	12,420	8,942	2,782	88
C.C.	Cmpd. F (free alcohol), 25 mg. over ½ hr.	0	10	8,750	5,722	2,048	344	18	7,200	4,032	2,405	344
		½	68	10,450	7,044	2,383	391	76	13,000	5,096	6,552	469
		1	46	12,350	8,077	3,013	356	52	11,750	5,499	5,006	416
	Epinephrine 1.5 mg. over 3 hrs.	2	35	12,850	9,818	2,184	234	34	16,575	12,365	3,580	156
		3	32	12,975	10,095	2,128	168	28	18,750	14,850	2,775	44
		4	14	13,300	11,013	1,676	156	21	17,300	14,913	1,522	28
		6	<2	14,450	10,880	2,818	175	11	17,400	14,129	1,844	32
		8	<2	15,025	10,688	2,855	328	8	16,175	12,358	2,944	88

The effects of intravenous epinephrine on the blood levels produced by intravenously administered steroids

The next approach was to determine whether epinephrine when administered with compounds E and F would alter the rate of utilization of the steroids, as judged by their rate of disappearance from the blood.

Normal males.—The data of the four experiments performed are presented in Table 4. No significant differences in 17-OH corticosteroid blood levels were observed. There was a marked cellular response to these doses of intravenously administered free steroid and a still greater change took place when the steroids were administered with epinephrine. At no time

TABLE 5. BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS AND LEUKOCYTES FOLLOWING INTRAVENOUS ADMINISTRATION OF COMPOUND E (FREE ALCOHOL), WITH AND WITHOUT EPINEPHRINE, TO A PATIENT WITH ADDISON'S DISEASE

Subject	Dose of steroids & epinephrine given, & length of time administered	Hours after start of infusion	17-OH steroids (γ/100 ml. of plasma)	Leukocytes (absolute number per cu. mm.)			
				WBC	PMN	Lymph.	Eos.
C.N.	Compound E (free alcohol), 50 mg. over 1 hour	0	<2	5,420	2,526	1,940	12
		1	44	4,860	2,284	1,798	6
		2	26	6,850	5,007	1,322	3
		4	14	7,120	5,625	1,253	3
		6	3	7,525	5,132	2,258	10
		8	<2	8,600	5,951	2,391	22
C.N.	Compound E (free alcohol), 50 mg. over 1 hour. Epinephrine, 0.75 mg. over 3 hrs.	0	<2	5,875	2,761	2,867	9
		1	44	7,700	4,312	3,018	25
		2	38	13,725	10,843	2,663	3
		4	18	11,850	10,523	995	3
		6	<2	11,475	9,089	1,997	0
		8	<2	9,700	6,926	2,192	6

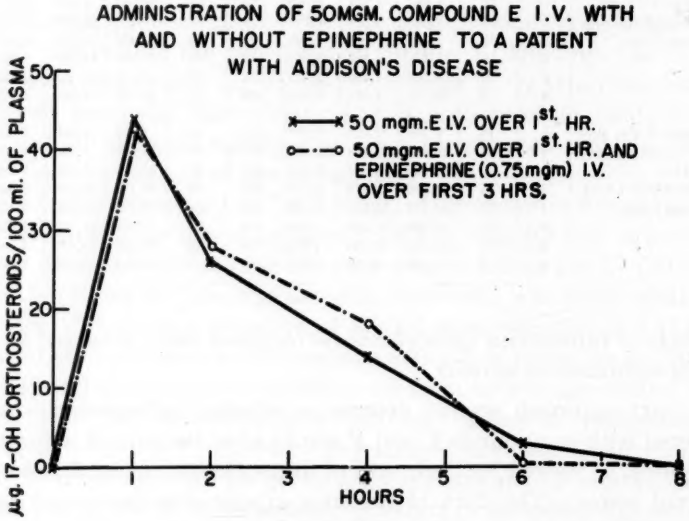

FIGURE 2

was there evidence that epinephrine changed the blood levels or the rate of utilization of administered steroids.

Addison's disease.—Table 5 and Figure 2 record the effect of the administration of 50 mg. of compound E (free alcohol), with and without epinephrine, to a patient with Addison's disease. It can be seen again that there was no significant difference in the rate of disappearance of 17-hydroxycorticosteroids from the blood. Special note should be made of the absence of detectable 17-OH corticosteroids in the control samples and eight hours after the administration of the steroids. Of interest also is the low level of eosinophils in this patient at the time of the experiments, for which we have no explanation. It is remarkable, however, that following several weeks of oral cortisone acetate therapy (12.5 mg. twice daily) the circulating eosinophils increased to 250 per cu. mm.

The effects of epinephrine on the rise in 17-OH corticosteroid blood levels produced by ACTH

These observations were made on 2 subjects who were given 25 units of ACTH intravenously over a period of two hours, with and without epinephrine. The data are presented in Table 6 and Figure 3. The most striking

TABLE 6. EFFECTS OF INTRAVENOUS ADMINISTRATION OF 25 MG. OF ACTH, WITH AND WITHOUT 1 MG. OF EPINEPHRINE, OVER A PERIOD OF TWO HOURS, ON THE BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS AND LEUKOCYTES OF NORMAL MALES

Subject	Hours after start of infusion	*ACTH (25 mg.) i.v. over 2 hrs.*					*ACTH (25 mg.) & Epinephrine (1.0 mg.) i.v. over 2 hrs.*				
		17-OH steroids (γ/100 ml. of plasma)	Leukocytes (absolute number per cu. mm.)				17-OH steroids (γ/100 ml. of plasma)	Leukocytes (absolute number per cu. mm.)			
			WBC	PMN	Lymph.	Eos.		WBC	PMN	Lymph.	Eos.
C.F.	0	21	6,240	2,009	3,108	679	12	6,550	2,424	3,210	691
	½	20	7,125	2,949	3,021	616	14	12,425	3,827	6,536	1,175
	1	24	7,355	3,972	2,309	538	16	11,600	3,758	5,870	1,262
	1½	22	7,990	5,114	1,774	525	18	11,850	4,977	4,953	1,172
	2	28	10,475	7,207	2,346	431	24	14,175	9,129	3,686	653
	3	40	10,500	7,245	2,772	188	25	18,800	16,243	2,030	168
	4	38	9,900	7,682	1,584	94	22	16,525	15,071	1,091	62
	6	15	10,025	7,679	2,045	38	8	14,900	12,635	2,056	62
	8	5	10,375	6,619	2,967	206	<2	14,600	10,424	3,621	326
A.D.	0	12	7,175	4,864	1,736	119	12	7,075	4,740	1,882	116
	½	17	6,325	4,225	1,720	84	17	12,625	7,534	4,065	247
	1	25	5,975	4,087	1,565	82	21	13,400	7,986	4,449	210
	1½	27	5,450	3,848	1,243	78	21	13,625	10,191	2,725	184
	2	33	6,825	5,201	1,283	69	19	17,025	13,450	2,656	144
	3	33	11,650	9,809	1,491	103	15	12,775	11,753	715	44
	4	25	10,225	8,671	1,329	72	18	12,525	11,348	952	25
	6	16	10,725	9,052	1,480	56	8	13,225	11,559	1,296	22
	8	8	10,775	8,167	2,177	66	<2	13,925	11,112	2,339	31

finding was the generally reduced level of 17-OH corticosteroids when ACTH was given with epinephrine. In contrast, the cellular changes were more pronounced when epinephrine was given with the ACTH than when the latter was given alone.

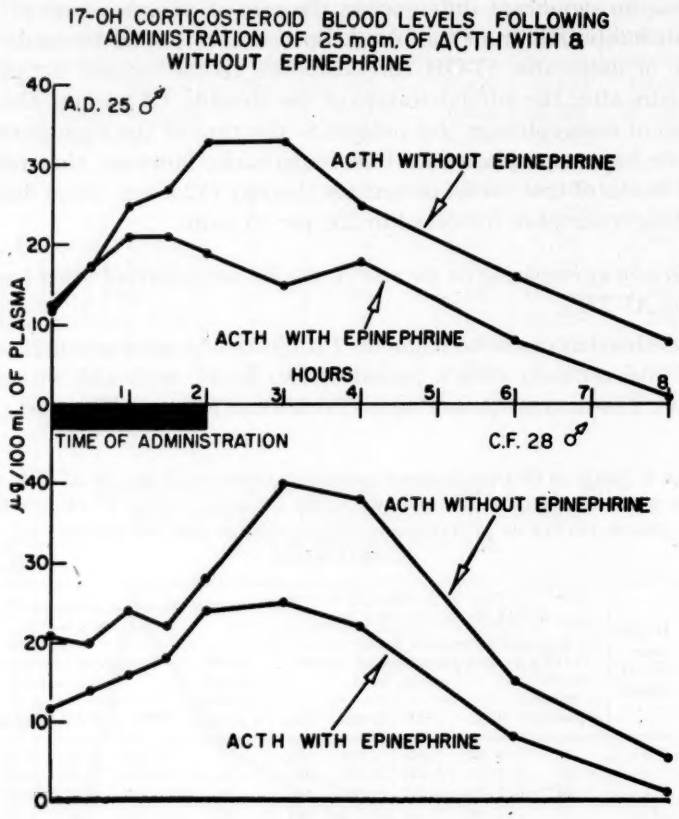

FIGURE 3

The effects of infusion of standard solutions without epinephrine or steroids

In order to determine what the administration of an intravenous infusion alone would do, 2 individuals were given intravenous infusions of saline and alcohol. The results are presented in Table 7. No rise in steroid levels occurred and the usual diurnal drop in 17-OH corticosteroid concentration took place in the late morning (37). No cellular changes of any great magnitude were observed.

TABLE 7. EFFECTS OF ADMINISTRATION OF FLUIDS INTRAVENOUSLY, WITHOUT CORTICOSTEROIDS OR EPINEPHRINE, ON THE BLOOD LEVELS OF 17-HYDROXY-CORTICOSTEROIDS AND LEUKOCYTES OF NORMAL MALES

Subject	Fluid given, & length of time administered	Time after start of infusion	17-OH steroids (γ/100 ml. of plasma)	Leukocytes (absolute number per cu. mm.)			
				WBC	PMN	Lymph.	Eos.
C.D.	Ethanol (10%) in 150 cc. of saline i.v. over ½ hr.	0 hr.	23	5,525	2,707	1,945	144
		½ hr.	16	4,725	2,240	1,833	100
		1 hr.	12	4,700	2,274	1,636	97
		3 hr.	7	5,775	2,737	2,298	100
		4 hr.	3	5,600	2,778	2,094	135
		6 hr.	5	5,225	2,602	1,923	100
		8 hr.	8	6,450	3,302	2,322	147
J.S.	Saline (250 cc.) with 10 mg. of heparin i.v. over 4 hrs.	0 hr.	9	8,450	4,918	2,163	469
		15 min.	7	7,200	3,989	2,131	438
		30 min.	6	7,850	4,459	2,340	400
		1 hr.	4	7,950	4,500	2,226	494
		2 hr.	<2	6,750	4,050	1,890	440
		4 hr.	<2	8,100	4,714	2,381	450
		6 hr.	8	6,900	4,085	1,960	384
		8 hr.	5	8,850	5,947	1,912	415

DISCUSSION

The foregoing experiments demonstrate that, following epinephrine administration, no increase in the concentration of circulating 17-hydroxycorticosteroids takes place; nor is epinephrine capable of modifying the rate of utilization of these steroids as judged by their rate of disappearance from the blood. The lack of any significant increase in the urinary output of 17-OH corticosteroids following epinephrine administration also indicates that no stimulation of the pituitary-adrenal system occurs.

Thus, these experiments neither give support to the view that epinephrine stimulates the anterior pituitary gland to produce ACTH (7, 12) nor do they favor the hypothesis that epinephrine stimulates the pituitary gland to produce ACTH through increased peripheral utilization of corticosteroids (4, 13). Finally, no evidence has been obtained to indicate that epinephrine stimulates the adrenal cortex directly to produce corticosteroids. The lack of any significant rise in adrenal cortical steroid blood levels after epinephrine in our experiments is in contrast to the findings of Vogt (14, 15) and of Paschkis *et al.* (38). These authors studied animals and used bioassay methods for the detection of adrenal steroid activity.

Several theories can be advanced to explain our results and those reported by other investigators.

1. *Epinephrine releases stored ACTH from the anterior pituitary gland without further stimulation of ACTH production*

It has been demonstrated that the intravenous administration of ACTH in amounts comparable to those present in the human adenohypophysis (39) results in a very significant elevation of 17-OH corticosteroid concentration in the blood (33). The cellular changes are also significant. As a matter of fact, the intravenous administration of as little as one-hundredth of the ACTH present in the human hypophysis (0.2 unit) results in a significant elevation of the 17-OH corticosteroid concentration in the blood (37). Since the administration of epinephrine failed to cause any significant elevation in the concentration of 17-OH corticosteroids in the blood, it would appear on first analysis that epinephrine does not cause the release of any significant fraction of stored ACTH. However, a recent publication suggests that epinephrine may decrease the quantity of ACTH in the anterior pituitary gland and in the peripheral blood of adrenalectomized animals (40). If this observation is correct, extra-adrenal metabolism of ACTH may be an important factor in the intact animal's response to epinephrine. Our data demonstrate only the over-all effect of epinephrine on circulating adrenal steroid and the metabolic activity of that steroid.

2. *Epinephrine increases release of ACTH by the anterior pituitary, but modifies the response of the adrenal cortex to ACTH*

Another recent study reports that epinephrine increases blood levels of ACTH in intact rats (41). There is some evidence from our experiments with ACTH and epinephrine given concomitantly, which indicates that there is indeed a decreased response of the adrenal cortex to ACTH in the presence of excess epinephrine. The mechanism of the modified response is obscure, although it may be due to vascular effects.

3. *Epinephrine stimulates the production of corticosteroids other than 17-OH corticosteroids*

There are several objections to this hypothesis. If stimulation of the adrenal cortex occurs via ACTH, an increase in 17-OH corticosteroids should have taken place, as ACTH causes an increased output of these steroids. If epinephrine stimulates the adrenal cortex directly to produce corticosteroids without the 17,21-dihydroxy-20-ketonic group, no changes in the cellular elements should have been observed. It has been shown that neither Kendall's compound A nor B is capable of producing the cellular changes seen following administration of 17-OH corticosteroids such as compounds E and F (42). Nevertheless, the production of hitherto undescribed corticosteroids by the adrenal cortex through epinephrine stimulation remains a possibility.

4. *Epinephrine potentiates the action of corticosteroids on peripheral cells, but neither stimulates the pituitary-adrenal axis nor increases the utilization of steroids*

It is possible that a synergistic action of epinephrine with corticosteroids already present in the blood or tissues may account for the cellular changes which follow epinephrine administration. Some evidence for this hypothesis is found in the experiments of Engel (44), as well as in our data. Engel has shown that certain of the metabolic effects of epinephrine do not take place in adrenalectomized animals, but do occur when the animals are pretreated with minute amounts of corticosteroids. Additional evidence for the synergistic effect of epinephrine and corticosteroids was published recently (44). The lack of change from the usual blood 17-OH corticosteroid levels in our experiments with epinephrine would point to the conclusion that epinephrine acts as a potentiator of the corticosteroids in their effect on peripheral cells.

If the last hypothesis is correct, then adrenalectomized patients who are maintained on 17-OH corticosteroids should manifest a normal cellular response to epinephrine. That such patients respond normally to epinephrine has been reported (30). It is also consistent that patients with Addison's disease but with some remaining functional adrenal cortical tissue would show a normal response to epinephrine. In such patients circulating corticosteroids would be present and their interaction with epinephrine would produce normal cellular changes. No further stimulation of the remaining adrenal tissue takes place when ACTH is given, because the endogenous amount of ACTH is more than enough to keep the small amount of adrenal cortical tissue functioning at maximal capacity. We believe that this explains the normal cellular response of certain Addisonian patients to epinephrine (20).

We have had the opportunity to check this hypothesis on a patient with adrenal insufficiency. Whereas most patients with Addison's disease, such as Subject C.N. in the experiments described in this report, have had no detectable 17-OH corticosteroids in their blood or urine, we have observed several Addisonian patients with low to normal 17-OH corticosteroid levels in the blood and urine. One of these was a patient with silico-tuberculosis who had signs of an Addisonian crisis which responded to the usual regimen. Intravenous ACTH tests (25 mg. given over a period of eight hours on two separate occasions) and several intramuscular ACTH tests failed to cause an increase in the blood levels of 17-OH corticosteroids and there was no response in the levels of eosinophils or other cellular elements. However, when the patient was given 0.5 mg. of epinephrine subcutaneously, a decrease in eosinophils of more than 50 per cent occurred in four hours, even though there was no significant change in the concentra-

tion of corticosteroids. This seems to indicate that the presence of corticosteroids, however small their concentration, is essential for the cellular effect of epinephrine but that the pituitary-adrenal system need not be intact.

Another interesting observation bearing on the above hypothesis is that of Recant *et al.* (20). These investigators gave aqueous adrenal cortical extracts to patients with Addison's disease who had previously shown no eosinopenia following epinephrine or ACTH. It was noted that one extract which produced physiologic changes suggesting that it contained small amounts of epinephrine, produced very significant decreases in eosinophils, whereas the other extracts were ineffective. The addition of small amounts of epinephrine to the latter extracts resulted in definite decreases in eosinophils. Thus, in our opinion, once again the synergistic action of corticosteroids and epinephrine on peripheral cells is suggested, an action not requiring a functioning pituitary-adrenal axis.

Some comments might be made concerning the effects of epinephrine on the cellular elements. The changes in leukocytes which follow epinephrine administration have been reported by previous observers (45, 47). Primary and secondary phases have been described. The former occurs within a half hour of the administration of epinephrine and consists of a leukocytosis due to an increase in polymorphonuclear cells and lymphocytes and to a lesser extent in eosinophils and other cellular elements. In approximately an hour the primary change is over. The secondary change in leukocytes occurs three to four hours after epinephrine administration and has been used as a test to measure the integrity of the pituitary-adrenal system (20). It consists of a leukocytosis (mainly polymorphonuclear), eosinopenia, and less frequently, lymphopenia. The eosinopenia and lymphopenia have been regarded as more reliable indices of pituitary-adrenal function than the polymorphonuclear leukocytosis, since the latter occurs in hypophysectomized and adrenalectomized animals and in patients with deficiency of pituitary and adrenal function. The data of our experiments show that the eosinopenia and lymphopenia which followed epinephrine were less marked than those which resulted from the administration of adrenal steroids or ACTH. The concomitant administration of epinephrine and adrenal steroids, or ACTH, produced more profound changes than those occurring when either was given alone, another fact pointing to a synergistic effect of these compounds.

SUMMARY

1. Epinephrine has been administered intravenously and subcutaneously in various doses and over varying periods of time to normal males, and the blood levels of 17-hydroxycorticosteroids as well as the urinary

excretion of 17-hydroxycorticosteroids and 17-ketosteroids have been measured.

2. Changes in blood 17-OH corticosteroid levels following epinephrine administration did not differ from the normal diurnal variations in these steroids. No increase in the urinary excretion of 17-hydroxycorticosteroids or 17-ketosteroids occurred after epinephrine administration.

3. Epinephrine did not influence the rate of disappearance of administered free cortisone or compound F from the blood, either in normal individuals or in a patient with Addison's disease.

4. The increase in 17-OH corticosteroids following concomitant intravenous ACTH and epinephrine administration was less than the rise following ACTH alone.

5. No evidence was obtained that epinephrine stimulates steroid secretion by the adrenal cortex or modifies peripheral utilization of steroids.

6. The most satisfactory explanation of our findings, in the light of present knowledge, is that epinephrine produces its adrenocortical-like cellular effects through a potentiation of the action of 17-hydroxycorticosteroids rather than through an effect on their secretion by the adrenal gland.

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THE EFFECT OF ACTH AND CORTISONE ON THE CONCENTRATION OF CIRCULATING ANTIBODY IN PATIENTS WITH ACUTE HEPATITIS*

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THE available information on the effects of ACTH and cortisone on patients with *chronic hepatic disease* suggests that these substances uncommonly have a beneficial action, whereas the frequency of untoward results emphasizes the hazards involved in their administration (1–6). In viral hepatitis, the use of these materials apparently alters certain manifestations of disease, often bringing about a sharp reduction in hyperbilirubinemia, and preliminary studies have suggested that they may exert beneficial effects in seriously sick persons (7–9). However, in the average case, the incidence of untoward side effects has also been stressed and the danger of relapse after too short a course of therapy is recognized (10).

In view of the apparently exaggerated effects of these substances or their metabolites in some patients with either acute or chronic hepatic disease, attempts were made to measure their action on the capacity of such patients to produce antibody. Previous investigations in this laboratory of the results of administration of ACTH or cortisone on the anamnestic response of patients with severe chronic hepatic disease, following the injection of purified diphtheria toxoid, revealed no alteration in the qualitative pattern of production of antitoxin when compared with patients who did not receive these materials. In addition, there was no limitation in the quantitative response, with the exception of the fact that

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when ACTH was administered as the amount of antitoxin was declining, there was a greater percentage of decrement of antibody when compared with the reduction found in control subjects not receiving these materials (11).

More recent studies of the potential of Schick-negative patients with acute viral hepatitis to produce diphtheria antitoxin following the intramuscular injection of highly purified diphtheria toxoid, revealed no significant difference in their qualitative patterns of production of antibody or in the intensity of immunologic response when compared with a group of essentially normal controls (12). In both groups, there was a considerable increase in circulating antibody by the seventh day after the injection of toxoid. The time of attaining the maximum measured response was variable, ranging from the second to the fifth week, with most persons reaching a peak on the fourteenth day. Following the attainment of a maximum amount of circulating antitoxin, there was usually a mild decrease during the next two weeks, although this did not always occur.

Since the potential for specific anamnestic response was apparently not changed in patients with acute hepatitis and in view of the alterations in the course of disease which are known to occur following the administration of ACTH or cortisone, it was believed to be of interest to determine the effect of administration of these substances on the capacity of such patients to produce antibody. It is the purpose of this paper to report the results of these experiments.

METHODS AND MATERIALS

Subjects. Ten Schick-negative patients with acute viral hepatitis, contracted while on duty with the U. S. Army of Occupation in and around Munich, Germany, during the summer months of 1951, were studied. The diagnosis was based on a characteristic history, signs of disease, and abnormality of certain tests of hepatic function. Purified diphtheria toxoid (50 Lf) was inoculated intramuscularly, and ACTH or cortisone was administered for varying periods in varying amounts. As a control, the amounts of antitoxin produced were compared with those made by another group of 34 young men with hepatitis studied during the same period of time, who had not received ACTH or cortisone (12). All the patients who received ACTH or cortisone had maximum measured amounts of serum bilirubin in excess of 5.0 mg. per cent and, as a group, they were somewhat more severely sick than their controls.

Antigen. Purified diphtheria toxoid[1] was used as antigen, and the anam-

[1] Furnished by the Division of Biologic Laboratories, Department of Public Health, Commonwealth of Massachusetts (courtesy of Dr. James A. McComb and Dr. H. J. Banton).

nestic response was measured in Schick-negative persons, since the production of antitoxin under these circumstances is far greater and follows a more predictable pattern than in the primary response of Schick-positive persons. Blood was obtained from all but 2 patients before Schick-testing. As a control for the Schick test, each person was also inoculated intracutaneously with 0.1 ml. (0.08 Lf/ml.) of diphtheria toxoid. Both reactions were read at the end of forty-eight hours, and persons who had negative results from the Schick tests and who had no pseudo-allergic reactions were inoculated intramuscularly with 50 Lf (0.14 mg.) of highly purified diphtheria toxoid. Blood was obtained subsequently on the seventh, fourteenth and twenty-eighth days after administration of the toxoid. Determinations of the amounts of antitoxin were made in each specimen.

Laboratory observations. The amount of diphtheria antitoxin in the serums was determined by titration[2] in the skin of rabbits (13), and all analyses were performed in duplicate. The serums used were frozen on the day of collection and were kept stored at dry-ice box temperature during the ensuing six months, when titrations were performed. The following determinations were made at least at weekly intervals and more frequently in the early part of the disease on all the patients with hepatitis: (a) total and one-minute direct serum bilirubin, (b) cephalin-cholesterol flocculation, thymol turbidity, and gamma-globulin turbidity, and (c) urinary bilirubin. The number of eosinophils in the peripheral blood was counted at appropriate times during therapy with ACTH or cortisone, and although a diminution in number of the order of 50 per cent or greater was found in 8 out of 10 patients, the reduction was frequently not sustained, even though the dosage of hormones was not altered. In the 4 patients (#1–4) whose dosage of ACTH was sharply reduced during the third and fourth weeks of therapy, the number of eosinophils increased promptly during this time.

EXPERIMENTAL

The effect of administration of ACTH or cortisone on the production of antibody when these substances were given at the beginning of hyperimmunization. Seven patients received ACTH (4 patients) or cortisone (3 patients) for periods ranging from sixteen to twenty-eight days, beginning within one hour after diphtheria toxoid was injected. The ACTH was administered intramuscularly in equally divided doses every six hours according to the following schedule: 100 mg. for two days, 80 mg. for two days, 60 mg. for

[2] Standard diphtheria antitoxin furnished by the U. S. Public Health Service, Biologics Control Laboratory, National Institutes of Health, Bethesda, Md. Standard diphtheria toxin furnished by the Division of Biologic Laboratories, Department of Public Health, Commonwealth of Massachusetts.

ten days, 40 mg. for seven days, 30 mg. for three to four days, and 20 mg. for three to four days. Cortisone was given intramuscularly in amounts of 100 mg. every eight hours for the first day, every twelve hours for the second day, and 100 mg. daily thereafter.

In Table 1 are recorded the amounts of circulating antitoxin found at regular intervals after the injection of toxoid. All but 1 patient (#1) made

TABLE 1. THE EFFECT OF ACTH OR CORTISONE ON THE CONCENTRATION OF CIRCULATING ANTITOXIN WHEN THE ADMINISTRATION OF THESE SUBSTANCES WAS STARTED IMMEDIATELY AFTER, OR FOURTEEN DAYS AFTER, THE INJECTION OF 50 Lf OF PURIFIED DIPHTHERIA TOXOID

Case* No.	Maximum measured serum bilirubin (mg. %)	Day of disease on which toxoid given	Therapy		Units diphtheria antitoxin/ml. serum before and on various days after injection with diphtheria toxoid			
			Days	Total dose (mg.)	Pre-Schick	7	14	28
			ACTH					
1	12.53	13	28	1,420	35.	27.5	22.5	27.5
2	10.10	12	28	1,420	7.5	37.5	75.	110.
3	14.50	11	28	1,420	—	17.5	32.5	15.
4	12.24	16	28	1,420	0.1	0.75	0.75	0.75
			CORTISONE					
5	7.59	6	28	3,100	0.75	12.5	32.5	27.5
6	7.88	5	28	3,100	0.75	45.	65.	27.5
7†	6.12	10	16	1,900	—	7.5	55.	55.
			ACTH					
8	10.58	18	21	1,240	5.7	7.5	7.5	7.5
9	6.24	56	17	1,080	0.4	2.	2.	2.
10	8.50	13	21	1,240	7.5	12.5	22.5	15.

* Patients #1–4 received ACTH for twenty-eight days following the injection of diphtheria toxoid. Patients #5 and #6 received cortisone for twenty-eight days, and Patient #7 for sixteen days following the injection of diphtheria toxoid. Patients #8–10 received ACTH starting the fourteenth day after the injection of diphtheria toxoid.

† This patient had a relapse of hepatitis after thirteen days of therapy with cortisone (*cf.* Fig. 2).

diphtheria antitoxin and, in some instances, in considerable amounts. It is possible that the large amount of circulating antitoxin (35 units/ml. serum—pre-Schick) present in Case #1 at the time of injection of toxoid may have neutralized the toxoid and made it ineffective as an antigen. Qualitatively, the pattern of response in the others was similar to that found in patients with acute hepatitis who did not receive ACTH or cortisone, *i.e.*, an increase by the seventh day, reaching a peak between then

TABLE 2. CORRELATION OF CLINICAL AND LABORATORY DATA AND THE AMOUNTS OF CIRCULATING DIPHTHERIA ANTITOXIN PRODUCED BY 34 PATIENTS WITH ACUTE VIRAL HEPATITIS, BEFORE AND ON VARIOUS DAYS AFTER THE INJECTION OF 50 Lf OF PURIFIED DIPHTHERIA TOXOID

Case No.	Severity of disease	Maximum measured serum bilirubin (mg. %)	Day of disease on which toxoid given	Diphtheria antitoxin (units/ml. serum)			
				Pre-Schick	7th day	14th day	28th day
11	moderate	3.6	13	—	12.5	47.5	47.5
12	severe	20.6	15	<0.1	0.6	7.5	8.2
13	mild	9.9	10	<0.1	0.1	4.	2.1
14	mild	4.2	14	<0.1	0.4	2.	4.4
15	mild	13.1	10	<0.11	0.1	0.4	0.1
16	mild	3.1	11	0.2	0.8	4.	4.
17	mild	5.2	21	0.2	1.41	7.8	7.8
18	moderate	11.4	10	0.2	0.2	0.4	0.4
19	mild	9.1	20	0.22	2.	12.5	12.5
20	mild	9.0	12	0.4	7.8	7.8	7.5
21*	severe	13.6	12	0.4	2.	8.2	7.5
22	moderate	4.7	14	0.4	23.6	42.5	32.5
23	mild	14.3	13	0.4	2.1	47.5	55.
24	moderate	8.0	14	0.82	4.	7.8	4.
25	severe	22.0	17	2.	7.5	13.1	7.5
26	moderate	11.7	15	2.	7.5	24.75	18.3
27	mild	7.5	19	2.	7.5	32.5	27.5
28	mild	10.9	9	2.	7.5	32.5	32.5
29	moderate	12.2	20	2.	—	3.5	2.
30†	mild	2.7	7	2.	13.1	17.5	17.5
31	mild	6.5	9	2.1	4.2	4.	2.1
32	mild	1.3	12	2.2	13.1	12.5	—
33	mild	4.4	12	2.2	26.2	49.5	60.75
34	mild	4.7	4	4.4	16.2	12.5	13.1
35	mild	4.2	13	7.5	17.5	23.6	21.7
36	moderate	15.6	12	7.5	4.	2.	2.1
37	moderate	12.8	12	7.5	85.	130.	65.
38	moderate	7.1	10	12.5	12.5	17.5	17.5
39	mild	4.8	15	13.1	110.	150.	—
40	mild	11.6	12	17.5	20.	28.8	22.5
41	moderate	5.3	12	23.6	23.6	28.6	28.8
42	mild	6.1	11	27.5	30.2	35.	32.5
43	mild	6.1	20	32.5	37.5	27.5	32.5
44	mild	8.1	15	42.5	51.75	51.75	47.5
Mean		*8.7*	*13.1*	*6.61*	*16.74*	*26.38*	*20.15*
Range		*1.3–22.*	*4–21*	*<0.1–42.5*	*0.1–110.*	*0.4–150.*	*0.1–65.*

* Secondary syphilis coincident with hepatitis. Treated intramuscularly during first ten days with penicillin.

† Relapse on eighteenth day after injection with diphtheria toxoid.

and the twenty-eighth day, followed at times by a decline after reaching a maximum (Table 2). Quantitatively, there was no apparent significant difference in the production of antibody between the patients who received ACTH or cortisone and similar untreated patients. The maximum measured amounts of antitoxin made by the 7 patients (#1–7) who received these materials for periods ranging from sixteen to twenty-eight days after the injection of toxoid averaged 46.2 units/ml. serum as compared with 28.4 units/ml. serum in the control group of 34 patients with hepatitis. However, because of the wide variation in amounts of antibody produced and the small number of patients in each group receiving ACTH or cortisone, it was impossible to assign any significance to this difference.

Only 1 of the 4 patients (#1–4) who received ACTH for twenty-eight days after the injection of toxoid had a decrease in the amount (54 per cent) of circulating antitoxin on the twenty-eighth day as compared to the amount present on the fourteenth day, whereas the 2 patients (#5 and 6) who received cortisone for a comparable period had reductions in circulating antitoxin of 15 per cent and 58 per cent, respectively, on the twenty-eighth day of therapy.

The effect of administration of ACTH or cortisone on the amount of circulating antibody when these substances were given after hyperimmunization was started. Three patients (#8–10) received ACTH for seventeen to twenty-one days according to the schedule just outlined, beginning on the fourteenth day after the injection of diphtheria toxoid. The determinations of circulating antitoxin are recorded in Table 1. All produced antibody following the injection of toxoid but only 1 out of the 3 had a decrease in circulating antitoxin (in the amount of 33 per cent) after fifteen days of therapy with ACTH.

Clinical status. A description of the effects of the administration of ACTH or cortisone on the clinical course of hepatitis in these patients will be included in another report (10). For the purpose of this study, it is sufficient to point out that certain previously observed alterations in the course of disease (7–10) caused by these substances were also found in these patients. Of particular interest is the fact that the decline in amount of serum bilirubin in the 6 patients whose serum bilirubin level measured 5 mg. per 100 cc. or more at the start of therapy was more rapid initially than was found, on the average, in a large number of other patients with viral hepatitis studied at the same time but not receiving these materials. In the other 4 patients whose serum bilirubin concentration measured less than 5 mg. per 100 cc. at the start of therapy, controlled studies were not made, but all but 1 case (#7) showed a rapid disappearance of jaundice. A moderate or marked eosinopenia developed in 8 out of 10 patients, although it frequently was not sustained. Glycosuria occurred at some time during the course of therapy in 9 out of the 10 patients; "mooning" of the face and

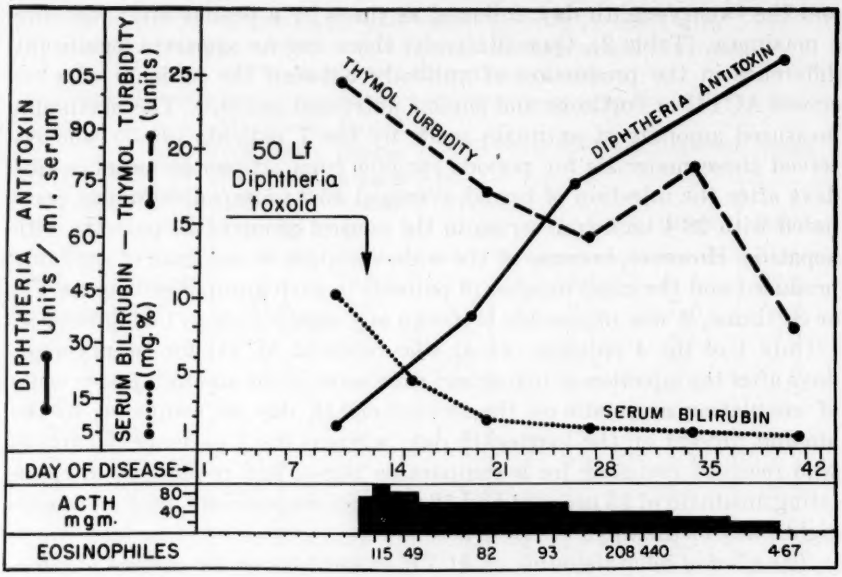

FIG. 1. Alterations in amounts of diphtheria antitoxin, serum bilirubin and thymol turbidity in a Schick-negative patient (#2, *cf.* Table 1) with viral hepatitis, following the injection of 50 Lf of purified diphtheria toxoid. ACTH was administered for twenty-eight days after the injection of toxoid.

retention of fluid were present in half the patients. However, in spite of the obvious changes effected, particularly in the initial rate of disappearance of jaundice, there was no apparent relationship between the severity of hepatitis, the alterations in the course of disease caused by ACTH or cortisone, and the amount of diphtheria antitoxin produced. In Figure 1 are recorded certain data on the course of disease of Patient #2, who was initially moderately sick, showing a rapid decrease in the intensity of jaundice associated with a great increase in the amount of circulating antitoxin during therapy with ACTH. In contrast are the data recorded in Figure 2 depicting the course of the disease of Patient #7 who initially was only mildly sick but who, after thirteen days of therapy with cortisone, had a relapse characterized by anorexia, nausea, and an increase in the concentration of serum bilirubin from 1.2 mg. to 6.2 mg. per 100 cc. However, in spite of the obvious worsening of his hepatic disease, he continued to produce considerable amounts of diphtheria antitoxin. Improvement in his disease was slow following cessation of therapy with cortisone, and mild histologic alterations, suggesting persistent activity of disease, were still demonstrable in a specimen of liver removed by biopsy 206 days after onset of disease.

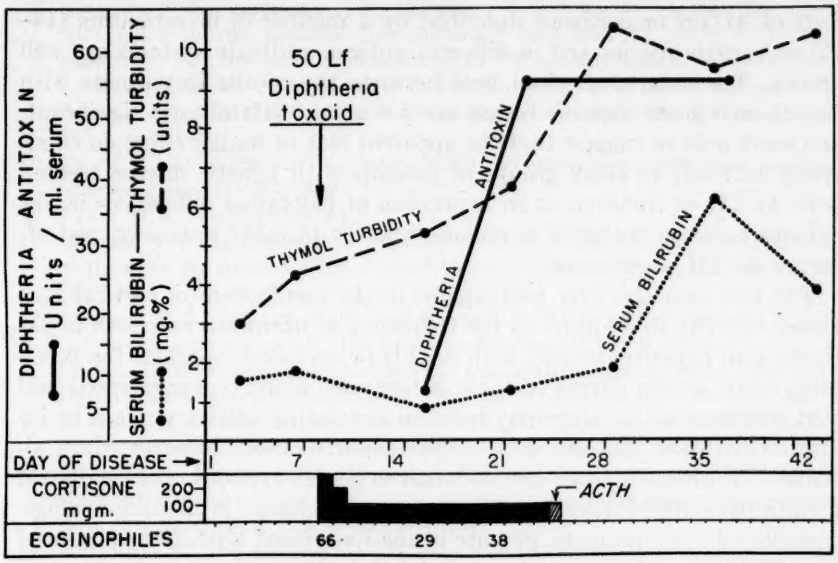

FIG. 2. Alterations in amounts of diphtheria antitoxin, serum bilirubin and thymol turbidity in a Schick-negative patient (#7, *cf.* Table 1) with viral hepatitis, following the injection of 50 Lf of purified diphtheria toxoid. Cortisone was administered for sixteen days and ACTH for one day after the injection of toxoid. This patient had a relapse of hepatitis after thirteen days of therapy with cortisone.

DISCUSSION

The data presented in this paper indicate that the administration of ACTH or cortisone to Schick-negative patients with viral hepatitis at various times after the injection of purified diphtheria toxoid apparently did not alter the qualitative pattern of anamnestic response or its intensity, when compared with the response found in similar patients not receiving ACTH or cortisone. These materials were given from the first day of hyperimmunization when the titer of antibody was increasing, and midway in the production of antibody when it had attained its maximum measured amount. These results are in general agreement with previous observations in this laboratory on the effect of ACTH or cortisone on the production of diphtheria antitoxin by Schick-negative patients with *chronic* hepatic disease, although certain quantitative differences are evident.

In the previous study (11) the administration of ACTH, beginning at about the time circulating antitoxin was at its maximum (fourteenth day), was associated with an increased decrement in the percentage of antibody, whereas the administration of cortisone during a comparable period did not have this effect. In the present study, the results of similar experiments tended to be exactly reversed. The apparently divergent ef-

fects of ACTH or cortisone described by a number of investigators (14–22) in various species and in different antigen-antibody systems are well known. The differences cited here between the results in patients with chronic and acute hepatic disease are not great, certainly not significant, and serve only to suggest that the apparent lack of similar effect on circulating antibody in small groups of patients with hepatic disease treated with ACTH or cortisone is an expression of individual differences in the balance between antigenic stimulus, capacity to make antibody, and effect of ACTH or cortisone.

The fact that the liver participates in the metabolism of cortical hormones (23–26), in addition to the frequency of untoward reactions in patients with hepatitis treated with ACTH or cortisone, suggests the possibility that certain effects of these substances might be exaggerated and that whatever action they may have on circulating antibody might be intensified in such patients. It is of particular interest, however, that, although the groups studied are too small to permit evaluation of significant quantitative differences, there was no obvious change in the immunologic capacity of these patients, in spite of the recognized alterations produced by ACTH or cortisone on the course of their disease.

SUMMARY

ACTH or cortisone was administered to 10 Schick-negative patients with acute hepatitis at varying times following the injection of 50 Lf of purified diphtheria toxoid, and the amounts of circulating antitoxin produced were measured by titration in the skin of rabbits. There was no significant alteration in the qualitative pattern of production of antibody or the intensity of immunologic response when compared with that of similarly immunized patients with viral hepatitis who did not receive ACTH or cortisone.

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COMPARATIVE OBSERVATIONS REGARDING UTILIZATION AND EXCRETION OF INFUSED GLUCOSE, FRUCTOSE, AND INVERT SUGAR, RESPECTIVELY*

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RECENTLY there has been renewed interest in the comparative utilization of glucose and fructose in diabetic and in nondiabetic subjects. The present study comprises observations in 11 nondiabetic subjects during and following the infusion of 1 liter of 10 per cent solution of glucose, of fructose, and of invert sugar, respectively.

METHODS

As indicated, the sugar solutions were infused at a reasonably uniform rate over a period of sixty minutes (plus or minus five minutes) in each instance. Blood was collected before the infusion was started, and at intervals of thirty, forty-five, sixty and ninety minutes after the commencement of the infusion. Total reducing sugars were determined in the blood by the method of Giragossintz *et al.* (1). Urinary sugars were determined on the specimen collected during the two-hour period following the commencement of the infusion.

RESULTS

In Table 1 and Figure 1 are shown the comparative results obtained in these 11 patients. Two things seem reasonably apparent from this study: first, that infused invert sugar and fructose disappear from the blood more rapidly than does glucose; and second, that this increased rate of disappearance is not referable to increased urinary excretion—in fact quite the contrary.

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TABLE 1. INDIVIDUAL VALUES FOR SUGAR IN THE BLOOD AND URINE OF PATIENTS RECEIVING GLUCOSE, INVERT SUGAR, AND FRUCTOSE, RESPECTIVELY

Patient	Blood sugar (mg./100 cc.)					Urine sugar (Gm. per 2 hrs.)
	0′	30′	45′	60′	90′	
Glucose infusion—(10% glucose in water)						
R. Gray	73	295	369	415	278	10.4
H. Edwards	53	229	218	214	58	5.6
J. Carroll	83	298	347	283	120	10.6
C. Wright	43	244	272	249	81	8.8
W. Kemp	81	313	365	407	269	13.9
Hallcraft	70	288	308	322	123	11.0
M. Jones	87	258	251	232	55	7.1
Bremer	43	—	267	176	54	13.2
Cruz	44	—	—	533	418	33.0
Jackson	82	229	307	309	229	7.1
McDaniel	73	137	208	197	266	1.7
Sum	732	2,291	2,912	3,337	1,951	122.4
Average	*67*	*255*	*291*	*303*	*177*	*11.1*
Invert sugar infusion(10% invert sugar in water)						
R. Gray	35	240	284	305	173	3.7
H. Edwards	48	162	147	144	35	2.7
J. Carroll	68	145	149	185	82	—
C. Wright	59	174	178	136	24	3.2
W. Kemp	76	152	172	—	199	—
Hallcraft	63	155	166	162	46	2.5
M. Jones	46	247	161	157	62	4.2
Bremer	75	260	303	264	147	7.7
Cruz	81	306	374	351	241	13.2
Jackson	66	180	233	268	—	1.4
McDaniel	72	282	274	249	184	1.8
Sum	689	2,303	2,441	2,221	1,193	40.4
Average	*63*	*209*	*222*	*222*	*119*	*4.5*
Fructose infusion—(10% fructose in water)						
R. Gray	52	175	194	162	54	5.2
H. Edwards	80	159	165	168	86	3.5
J. Carroll	84	150	186	199	105	3.8
C. Wright	48	115	133	130	103	3.6
W. Kemp	88	232	208	232	124	—
Hallcraft	51	166	171	144	61	5.2
M. Jones	75	161	162	155	98	5.1
Bremer	86	119	152	174	98	7.4
Cruz	79	258	271	242	120	14.7
Jackson	59	167	—	176	80	10.7
McDaniel	—	118	—	135	116	.8
Sum	702	1,820	1,642	1,917	1,045	60.0
Average	*70*	*165*	*182*	*174*	*95*	*6.0*

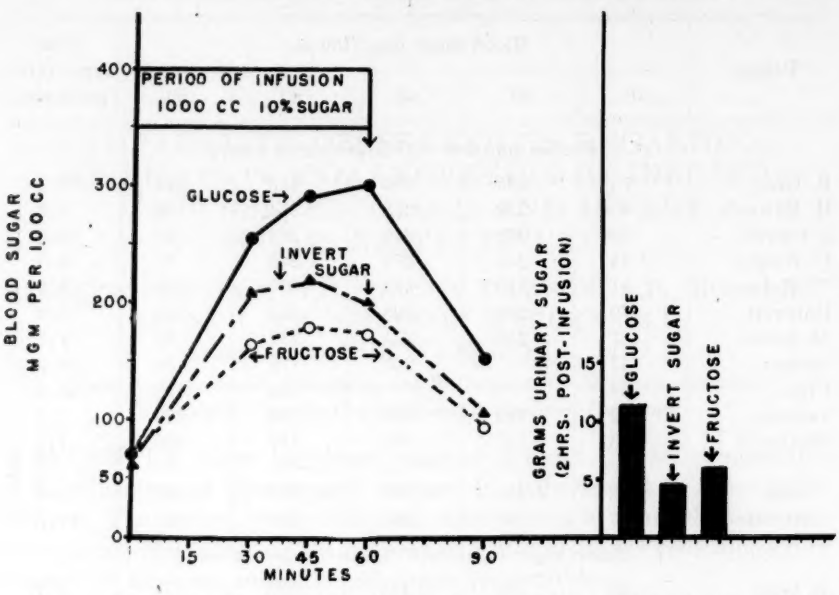

FIG. 1. Average levels of total reducing blood sugar during and following the infusion of glucose, invert sugar, and fructose, respectively, and of urinary sugar during the two-hour period following such infusions.

DISCUSSION

The blood sugar findings in patients receiving fructose and glucose are in accord with those reported by Weichselbaum, Elman and Lund (2). On the basis of the blood and urinary findings, it appears that the differences in the rate of disappearance from the blood stream must be referable to increased utilization. Whether this utilization is referable to an increased rate of oxidation, to an increased rate of glycogen formation, or to an increased rate of formation and storage of fat, is at the present time unknown. Respiratory quotient studies and the use of isotopically labeled material will be employed in the near future to evaluate these questions more precisely.

In some instances the invert sugar appeared to be utilized at a more rapid rate than did fructose. This finding would be compatible with the concept that glucose and fructose are utilized through independent pathways. Such concurrent utilization, on occasion, could result in a more rapid total utilization of invert sugar than of fructose per se.

SUMMARY

Invert sugar and fructose when infused intravenously in nondiabetic

subjects are utilized at a significantly more rapid rate than is glucose. As a corollary, there is less glycosuria.

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THE EFFECT OF INTRAVENOUS ACTH ON THE BLOOD LEVEL OF ASCORBIC ACID AND OF SULFHYDRYL AS DETERMINED BY A MODIFIED AMPEROMETRIC TECHNIQUE*†

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DURING the early nineteen-thirties, considerable interest was aroused by the report of an unusually high concentration of sulfur in the adrenal cortex (1); presently, glutathione, dimethyl sulfone, taurine, bis-β-hydroxy ethyl sulfoxide and ethyl hydrogen sulfate were identified in this tissue (2). The physiologic implications of these data remained obscure, but their importance was increased by the observation that percutaneous administration of colloidal sulfur caused marked storage of sulfur and cellular hypertrophy of the adrenal cortex, as well as deposition of glycogen in the liver (3).

A renewed interest in the relation of sulfur to the adrenal cortex has arisen from an entirely unrelated problem, namely the investigation of alloxan diabetes. Grunert and Phillips (4), in the course of such studies, observed a lowered concentration of "blood glutathione" in sodium-depleted rats. They noted a correlation between these findings and those of Binet and Pautonnet (5), who found in Addisonian patients a lowered "blood glutathione," which was restored to normal by therapy with desoxycorticosterone acetate. Conn and his group (6), studying the derangements of carbohydrate metabolism during ACTH administration and mindful of the problems of alloxan diabetes with regard to sulfhydryl, observed depression of the "blood glutathione" level during such treatment, as well as in the course of cortisone or compound F therapy. Kass, Ingbar

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and Finland (7) observed in 1 case a rise in the "blood glutathione" level after interruption of ACTH administration. Sprague *et al.* (8) found little change in the "blood glutathione" of 3 patients receiving ACTH, and a slight increase during administration of cortisone. Hess, Kyle and Doolan (9) studied 11 patients treated with cortisone and 11 treated with ACTH; the former group showed a drop in the "blood glutathione" level which was statistically not significant, whereas the ACTH group showed a significant depression ranging from 5 to 48 per cent of the control level. Levin and associates (10) found a decrease in the "blood glutathione" of 1 patient during the first course of ACTH therapy but no changes during subsequent treatment. Likewise, Henneman and Altschule (11), treating 4 psychiatric patients with ACTH and glucose, found a rise in "blood glutathione" in 3 and no change in the fourth. Similar observations were made in other psychotic patients subjected to the stress of epinephrine, insulin treatment, or electroshock therapy. Ingbar, Otto and Kass (12) found no change in the blood sulfhydryl level of rats treated with substantial doses of ACTH for periods of from three to ten days.

Kinsell and co-workers (13) have observed a 50 per cent rise in the urinary sulfhydryl-plus-disulfide sulfur during ACTH therapy.

Various hypotheses have been advanced to explain the relationship of sulfhydryl and adrenal cortical function. Conn (14) has postulated that the reversal of certain ACTH effects by the intravenous administration of glutathione is due to "improved performance of those systems which require free sulfhydryl groups for their normal function. This appears to be the case with respect to 1) renal tubular reabsorption of glucose; 2) the systems involved in the utilization of glucose, and 3) the systems responsible for the production and release of white cells." Other workers have thought for some time that glutathione serves as a reactivator of SH enzymes generally, and recently Anderson *et al.* (15) have made the converse suggestion that ACTH exerts many of its metabolic and clinical effects by causing SH depletion and hence interference with those enzyme systems which require free SH groups for their activity, such as succinic dehydrogenase, pyruvate oxidase, systems requiring coenzyme A, and others.

We have entertained the hypothesis that glutathione and ascorbic acid, which occur together in the adrenal cortex in relatively high concentrations, may form an oxidation-reduction system of some sort, possibly concerned with the enzyme processes which elaborate the oxygenated cortical steroids. Many others have also been aware of the possible relation between glutathione and ascorbic acid: the question of a peripheral reaction involving oxidized glutathione and ascorbic acid in equilibrum with reduced glutathione and dehydroascorbic acid, for example, has been investigated recently by Anderson *et al.* (15). In these studies, the intra-

venous administration of 1 to 5 grams of ascorbic acid failed to influence "blood glutathione" levels (by the iodate method). These results are not in agreement with the earlier work of Prunty and Vass (16) who gave massive oral doses of ascorbic acid and found 18 to 26 per cent depression of the "blood glutathione" by the same technique.

That large doses of ascorbic acid influence adrenal cortical function seems fairly clear in view of animal experiments (17) and clinical investigations (18, 19), including recent studies (20) by one of us (J.W.G.). However, the nature of this relationship between sulfhydryl and ascorbic acid, and the site of its control remain obscure.

In the course of studies on the relation of adrenal cortical function to sulfur metabolism, we investigated the simultaneous effects of ACTH on blood ascorbic acid and blood sulfhydryl levels with a more refined technique and under conditions which might yield less equivocal results than are reported in the current literature.

MATERIALS AND METHODS

Fifteen patients without any severe or chronic stressful process were chosen (custodial cases of chronic varicose ulcers, ambulatory simple-fracture patients ready for discharge, patients with mild coronary occlusion at the end of the period of convalescence). In the fasting state, blood was drawn for hematocrit, ascorbic acid and sulfhydryl determinations; then an infusion of 500 ml. of isotonic saline was administered over a period of eight hours. On succeeding days, 10 I.U. of ACTH was dissolved in the infusion fluid. The ACTH was administered in this manner on one to three successive days, for eight hours each day.

Hematocrit and blood ascorbic acid values were determined by standard methods (21, 22).

The determination of blood sulfhydryl must be described in some detail, as we have made further modifications of the original method of Kolthoff and Harris (23), already altered by Benesch and Benesch (24) and by Weissman, Schoenbach and Armistead (25). The method is based on the principle that, in alcoholic ammonia solutions of high pH, the sulfhydryl radical (SH) will still combine with silver ion, forming undissociated silver mercaptide (AgSH), in contrast to other ions present. When an excess of silver ion is present, the solution becomes conducting in direct proportion to the concentration of free silver ions. A potential applied across the solution will cause a current to flow, and this may be measured by a galvanometer.

We were unable at first to obtain adequate reproducibility and found that Weissman *et al.* had also experienced considerable difficulty with the response and stability of platinum electrodes. After much experimentation,

a procedure was evolved which we believe is more specific for SH than methods previously employed, and is consistent and reproducible within 2 per cent. It should be emphasized that the method measures *free sulfhydryl groups only*, whether they occur as glutathione or otherwise.

The few items of special glassware (mercury cell, sintered glass electrode) were prepared according to the directions of Kolthoff and Harris by the Emil Greiner Co. of New York. An outline of the apparatus is given in Figure 1. A large-bore stopcock is inserted into the rubber tubing between the mercury cell and the glass electrode. The cell is layered with mercury and filled with electrolyte, which consists of 4.2 Gm. of potassium iodide and 1.3 Gm. of mercuric iodide per 100 ml. of saturated potassium chloride solution. The glass electrode is filled with a hot solution of 3 per cent agar and 30 per cent potassium chloride, and allowed to cool. The liquid bridge (6–8 mm. rubber tubing) connecting the two is filled with saturated potassium chloride, taking great care to avoid air bubbles. A T-tube can be incorporated into the liquid bridge in order to simplify this procedure. The rotating platinum electrode, made according to the direc-

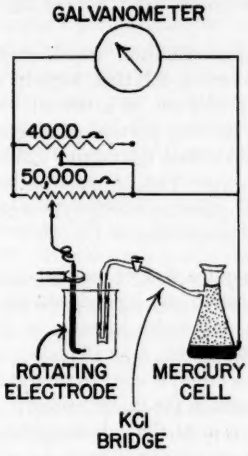

FIGURE 1

tions of Kolthoff and Harris, is of 19-gauge platinum wire or larger, and should project half an inch beyond the glass. The rotating electrode is held in a pulley support (E. Greiner Co., cat. no. G22840) and mounted on a ringstand. The pulley is connected by a small belt to the reduction gear of an ordinary stirring motor (E. Greiner Co., cat. no. G22745). A rheostat to control the speed of rotation, and a constant voltage transformer are placed in the 110-volt line of the motor. A rotation speed of about 120 r.p.m. is convenient; there is not sufficient variation in speed with this arrangement to affect the output current. Both electrodes are adjusted to fit into a 250-ml. Pyrex beaker, which serves as the reaction vessel.

The galvanometer used was of much higher sensitivity than those previously employed. We used a G.E. galvanometer, type 32C-243-G9 with a sensitivity of 0.0032 microamps. per scale division and a critical damping resistance of 3,450 ohms. An electrical circuit involving two variable resistors (such as may be purchased at any radio

supply store) is introduced as shown, to permit variation of the critical damping resistance and of the total resistance of the external circuit. By this means it is possible to alter the sensitivity of the circuit at will, or in other words, to compensate for changes of external resistance or electrode response, or for replacement of the rotating electrode with another one of different characteristics. Previously, selection of a suitable electrode involved the preparation of a considerable number; the selection of one with adequate characteristics was made by trial and error. With the circuit employed, it is possible to maintain a constant electrode sensitivity of 20 galvanometer scale units deflection per 0.1 ml. of 0.001 N silver nitrate solution. The constant-voltage transformer, rheostat, variable resistors, switches, and so on are mounted on a standard metal radio panel for convenience.

Reagents:

1. Saponin (0.01 per cent in distilled water) as a laking agent.
2. Sulfosalicylic acid (25 per cent in distilled water) as a protein precipitant.
3. Disodium ethylenediamine tetraacetate (EDTA), 0.335 per cent in distilled water. This is a complexing agent which stabilizes the SH in solution.
4. Sodium *p*-chloromercuribenzoate, 0.001 N (346 mg. per liter), as a specific SH-removing agent.
5. Exactly 0.100 N silver nitrate solution, which may be checked against standard sodium chloride (26). This stock solution, kept in a brown bottle in the dark, is made up to 0.001 N just before use, in a 100-ml. volumetric flask.
6. Supporting electrolyte. A 30-Gm. portion of reagent grade ammonium nitrate is added to a 250-ml. volumetric flask containing 125 ml. of concentrated ammonium hydroxide and 10 ml. of water. This is made up to volume at room temperature with distilled water. The supporting electrolyte is prepared by adding this reagent to absolute ethanol in the proportion of 1 to 29.

Preparation for titration: The outer shell of the stationary glass electrode is filled with saturated potassium chloride solution and slipped into place.[1] Next, 30 ml. of supporting electrolyte, dispensed from an automatic burette, is placed in the beaker, and the motor of the rotating electrode is started. A small glass pipette, U-shaped at one end and with a rubber suction bulb at the other, is used to aspirate any air bubbles in the bottom of the glass electrode. The stopcock in the liquid bridge is opened, completing the circuit, and the system is allowed to run until the galvanometer is stable, usually from five to ten minutes. A trial run to test sensitivity is made by adding 0.1-ml. portions of silver nitrate solution to the electrolyte in the beaker. There should be a 20-scale-division deflection per aliquot. When these things are in order, the beaker of solution is removed, the electrodes washed off with supporting electrolyte from a wash bottle, and a new beaker of supporting electrolyte put in place. The equipment is then ready for use. It is advisable to check the electrode against a standard sulfhydryl solution. We were unable to obtain the *n*-dodecyl mercaptan used as a standard by Weissman *et al.*, and found that cysteine, α-mercaptoacetanilide and a number of other mercapto compounds were unsuitable. Finally we used a freshly prepared solution of glutathione (9.30 mg. per 100 ml.) in water with a small amount of EDTA, and found it to be stable for about forty-eight hours. This solution contains 1.0 mg. sulfhydryl per 100 ml.

Preparation of the blood filtrate: Exactly 4.0 ml. of oxalated or heparinized blood is

[1] The rubber washer between the two parts should fit tightly; otherwise the potassium chloride will leak into the supporting electrolyte, precipitate in the solution and on the surface of the platinum wire, and thereby change the response characteristics.

added to 14.0 ml. of saponin solution. This is allowed to stand with occasional shaking for five minutes. Then 2.0 ml. of sulfosalicylic acid is added with shaking, the solution is allowed to stand for another five to ten minutes, and filtered. A slight cloudiness does not interfere with the titration. This solution is stable for several hours.

Titration: The rotating electrode, which has reached stability in 30 ml. of supporting electrolyte, should show no permanent deflection while 0.2 ml. of EDTA and 1.0 ml. of filtrate are added to the beaker. After a few moments, 0.1-ml. portions of silver nitrate are added from a pipette or microburette at about sixty-second intervals and the galvanometer readings noted. When the deflection has exceeded 80 units, titration is stopped, the beaker removed, the electrodes rinsed with electrolyte, and a fresh beaker placed in position.

The galvanometer readings are now plotted as the ordinates against the volume of silver nitrate as abscissa, on rectangular coordinate paper. The linear portion of the curve is then extrapolated down to the zero ordinate and the volume of silver nitrate read off the abscissa. Duplicates should agree within 2 per cent, even though the slopes of the runs may differ.

The *blank* is now determined by adding to the supporting electrolyte 0.2 ml. of EDTA, 1.0 ml. of filtrate, and then sodium *p*-chloromercuribenzoate solution in an amount 0.1 ml. greater than the amount of silver nitrate read off the curve of the previous titration. The chloromercuribenzoate specifically removes all the SH groups, hence the silver now titrates any nonspecific silver-removing substances which may be present. The titration proceeds in the same manner and the calculation is identical. The amount of silver nitrate required in this run (in duplicate if necessary) is subtracted from that of the original run, giving as the difference the volume of 0.001 N silver nitrate required exclusively to remove all the SH contained in 1.0 ml. of filtrate (0.20 ml. blood). The 0.001 N silver nitrate is equivalent to SH in a concentration of 3.307 mg. per 100 ml.; in other words, 1.00 ml. of 0.001 N silver nitrate is equivalent to 33.07γ of sulfhydryl.

After the *p*-chloromercuribenzoate blank has been determined, it is well to allow the electrodes to run for a minute or two in supporting electrolyte containing some cysteine. This neutralizes any excess chloromercuribenzoate present. Subsequently, the usual rinsing with supporting electrolyte is carried out, and the instrument is ready for the next titration.

Calculations are very simple. One must correct for the dilution of the filtrate (5×), for the electrode standardization factor found with glutathione, and for the hematocrit of the blood. As nearly all the nonprotein sulfhydryl of the blood is contained in the red cells, omission of this hematocrit correction (which is common) may lead to entirely misleading results. The final answer may be expressed either as milligrams of sulfhydryl per 100 ml. of packed red cells or as milligrams of sulfhydryl per 100 ml. of blood, corrected to a hematocrit of 50 per cent (and so noted).

RESULTS

Presentation of data. By the method employed, the average blood SH concentration was about 2.70 mg. per 100 ml. of blood (with a 50 per cent hematocrit). However, as the individual variation was considerable, our results were treated not in terms of absolute values, but as percentile changes from the control level, in the manner usually employed for the eosinophil count in measuring adrenal function. Hematocrits showed no significant change during the infusion period. Blood ascorbic acid values

were also expressed as percentile changes from the control value, for the sake of uniformity.

Alterations in blood SH concentration. The percentile change in the blood SH level after four hours' infusion is shown in Figure 2. The first column indicates the effect of a control infusion of saline alone, and the succeeding columns the effect of ACTH (a total of 5 mg. at the time blood was drawn) on successive days. After four hours of control (saline) infusion, 1 patient showed an 8.5 per cent depression of the blood SH level, whereas the other 4 showed rises up to 23 per cent in magnitude. By contrast, after 5 mg. of ACTH had been infused on the first day, 10 of 12 patients showed a *drop* in

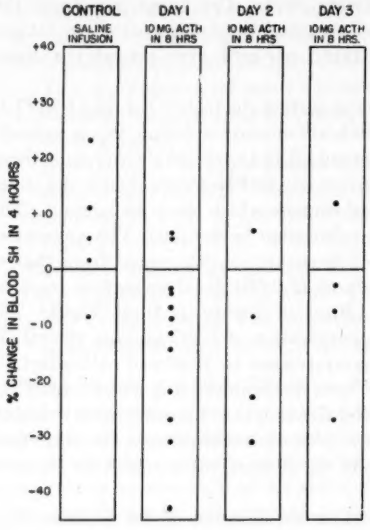

FIGURE 2

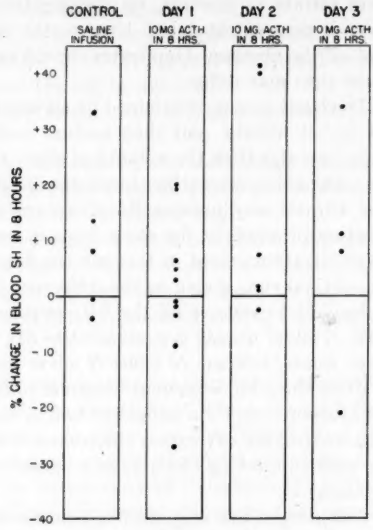

FIGURE 3

level ranging from 3.5 to 43 per cent. There are fewer data for the second and third consecutive ACTH infusions, but in both instances 3 out of 4 patients showed a decline in the blood SH level.

The picture changed after eight hours of infusion as compared to the control level (Fig. 3). With saline infusion alone, there was little change from the findings noted at four hours. However, at the end of the first ACTH infusion, a number of the previously depressed values returned to the baseline level (in 7 of 10 patients they were within 10 per cent of the control value). One remained depressed 27 per cent below the original level, and 2 rose 20 per cent above the control value. Similar changes also occurred on the second and third day of ACTH infusion, for on both these days the eight-hour levels were considerably above the four-hour depres-

sion, and in most cases had returned to or exceeded the control level. One may conclude, then, that during the continuous intravenous infusion of ACTH at the rate of 1.25 mg. per hour, there is a transitory depression of the blood SH level, detectable at four hours, and there is then a return toward normal levels within eight hours despite continued infusion.

Alterations in blood ascorbic acid level. After four hours of control (saline) infusion, the changes in blood ascorbic acid level were quite variable, with 3 cases showing an elevation and 1 a depression (Fig. 4). In contrast to the effect on blood SH levels, four hours of ACTH infusion had little effect the

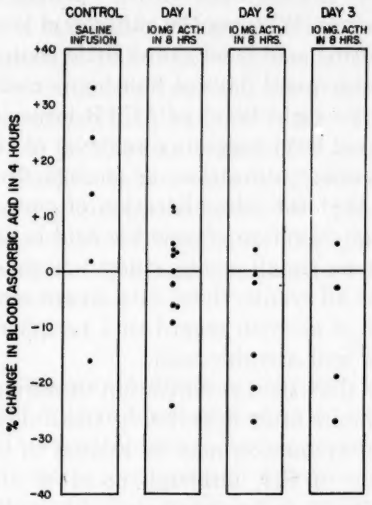

FIGURE 4

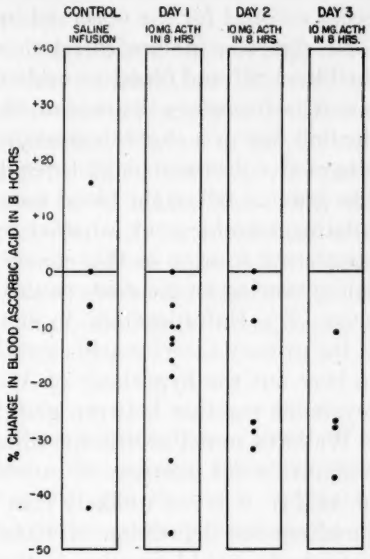

FIGURE 5

first day: all but 1 of the patients showed an ascorbic acid level within 12 per cent of the control value. On the second and third days of ACTH infusion, a tendency toward lowered values appeared.

After eight hours of control saline infusion (Fig. 5), there was again a wide scatter of results with no definite alteration from the initial values. During all three days of ACTH infusion, however, a depression from the original level was evident in most cases, and no actual elevations were noted.

DISCUSSION

From the data just presented it would appear that, with fair consistency, intravenous infusion of ACTH will produce a transitory depression of the

blood SH level. The return to the control SH level despite continued infusion is surprising, and we have no explanation to offer at the present time. This phenomenon might have been overlooked if only a single intramuscular injection of ACTH had been given. Furthermore, it is significant that the transitory depression is reproducible on consecutive days, so that no "resistance" to this effect of ACTH seems to develop in the interval studied. The characteristics of this response to ACTH may possibly explain some of the contradictory published observations on patients treated with this substance; if the effect of ACTH is also transitory when intramuscular injections are given, then neglect of this time factor may easily account for the observed inconsistencies.

No direct or inverse correlation seems to exist between the changes in the blood SH and blood ascorbic acid levels. Whereas the sulfhydryl level reveals a transitory depression, the ascorbic acid level shows little change the first day or a slight depression on subsequent days at four hours and a progressive decrease on all three days after eight hours of ACTH infusion. The gradual fall of the blood ascorbic acid level suggests a removal of circulating ascorbic acid, whether by excretion, utilization, or storage. It is interesting to note in this connection, that the administration of certain sulfur-bearing amino acids causes a large excretion of ascorbic acid in the urine (27). Unfortunately, in our work no simultaneous study was made of the urinary ascorbic acid excretion. At all events, these data do not seem to bear out the hypothesis of Anderson *et al.* with regard to a peripheral reversible reaction between glutathione and ascorbic acid.

We have noted in other studies (28) that the concentration of adrenal nonprotein SH increases in rats three hours after injection of small doses of ACTH; it is not unlikely that this phenomenon may be related to the simultaneous depression of blood levels of SH, although proof of this hypothesis would require further studies, as for example with radioactive S^{35}. In considering this hypothesis, one cannot ignore the fact that results from two different species and two different modes of ACTH administration are being considered.

It seems timely to comment upon the current procedures for the measurement of sulfhydryl groups, and the terminology involved. The usual methods depend on titration with oxidizing agents, iodine being most commonly employed. All these methods suffer from two distinct disadvantages. In the first place, they are not specific for SH, as a number of other substances (particularly ascorbic acid) are likely to interfere. This fact, for example, renders iodine methods useless for the determination of SH in adrenal tissue. With iodine, furthermore, there is the problem of a dilution effect, that is, the dependence of the result on the iodine:glutathione ratio, owing to oxidation beyond the disulfide stage (29). Despite the fact that

normal blood values obtained by iodine methods agree reasonably well with those obtained by the amperometric method, there is no assurance that such agreement would continue under clinical or experimental conditions which may involve changes in the nonspecific reacting substances.

Furthermore, blood values have been generally reported without regard to the hematocrit. As nearly all the nonprotein sulfhydryl is contained in the red cells, the influence of hemodilution or hemoconcentration is of paramount importance, particularly in studies on cortisone-treated or ACTH-treated patients, in whom changes in blood volume are known to occur. It is questionable whether data which have not taken this factor into account can be meaningfully interpreted.

Lastly, there is the question of terminology. Most authors report results in terms of "blood glutathione." The fact remains that the glyoxalase technique (30) is the only one which measures glutathione specifically, and this manometric method is not usually employed. Whether or not the major component that is being measured happens to be glutathione, it hardly seems justified to report the results in terms of glutathione when the method measures only oxidation-reduction capacity in most instances. Even in the method described here, which we believe to be more specific than previous techniques (except glyoxalase), only the free sulfhydryl group is being measured, and we feel that results should be reported in these terms.

SUMMARY

Changes in blood sulfhydryl levels during intravenous infusion of ACTH have been studied in 15 individuals. The changes were compared with those obtained during infusion of saline, and showed a consistent drop after four hours of infusion. This alteration could be reproduced on successive days in the same patient.

The blood sulfhydryl level after eight hours of infusion (10 mg. of ACTH) showed a return to the control value in most cases, revealing that the depression of the SH level during ACTH infusion is transitory.

Determination of blood ascorbic acid concentration in the same patients showed a progressive and not a transitory depression.

A technique of amperometric titration of SH groups, based on the method of Kolthoff and Harris, is described. The method is more specific for SH groups, and eliminates technical difficulties previously encountered at low concentrations of sulfhydryl.

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FRACTIONATION BY COUNTERCURRENT DISTRIBUTION OF URINARY ESTROGENS IN NORMAL INDIVIDUALS AND IN PATIENTS WITH HYPERADRENOCORTICISM*

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IN ESTABLISHING the pattern of urinary estrogens which probably originate from the adrenals, it seemed particularly important to determine whether it differed in untreated cases of adrenal hyperplasia and tumor from that in normal women and men. Studies were made on 2 normal women, 1 pregnant woman, 2 normal men, 2 patients with virilizing adrenal tumor and 4 patients (2 males, 2 females) with congenital adrenal hyperplasia before and during cortisone therapy.

The present methods of determining urinary estrogens are unsatisfactory because estrone, estradiol-17β and estriol[1] have widely different biologic, fluorometric and colorimetric potencies. To study the excretion of urinary estrogens satisfactorily it is necessary to employ fractionation methods. In the present work the countercurrent distribution technic of Craig and co-workers (1), as adapted to estrogen fractionation by Lewis L. Engel and colleagues (2), was employed. To obtain good separations, however, it was found necessary to make several redistributions, employing different systems of solvents to be described.

METHODS

Collection and extraction procedure: Twenty-four-hour specimens of urine were collected in bottles containing 15 ml. of 25 per cent acetic acid and stored in a cold room. The method of hydrolysis and extraction described by Engel and colleagues (2) was followed, except that the 1N NaOH extract was brought to pH 7 instead of pH 9. Ether and toluene (Merck) were redistilled before being used. In normal individuals a 72-hour urine collection, or a larger amount, was extracted. A smaller extract can be used when

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† Fellow of the André et Bella Meyer Foundation (France), on the recommendation of the Institute of International Education, New York.

[1] The nomenclature used in this paper is that advised by Fieser and Fieser (21).

the estrogen level is expected to be high, although it is better to use the largest amount available.

Countercurrent distributions: These were carried out in the 25-tube stainless steel instrument described by Craig and Post (3). The technic is described elsewhere (4). The following systems of solvents were used:

System I = 50 per cent methyl alcohol/carbon tetrachloride

System II = 50 per cent cyclohexane—50 per cent ethyl acetate/33 per cent ethanol

System III = 60 per cent methyl alcohol/carbon tetrachloride

After distribution, the total content of each tube was pipetted into a separate flask and was evaporated to dryness on a steam bath.

Photofluorometric analysis: The estrogen residue was dissolved in a measured volume of redistilled absolute ethyl alcohol. Two-tenths ml. of the alcoholic extract was added to 1 ml. of concentrated H_2SO_4 and put in a boiling water bath for twenty minutes,

TABLE 1. RECOVERY OF ESTRONE, ESTRADIOL-17β AND ESTRIOL ADDED TO ACID SOLUTION BEFORE AND AFTER AUTOCLAVING

(The recovery was determined at the end of the extraction procedure and after countercurrent distribution. The autoclave neither destroyed the estrogens, nor transformed one of them into another compound)

Method	Estrogen used	Per cent recovery	
		Determined at the end of extraction procedure	Determined after countercurrent distribution
Standard added before autoclaving	Estrone	80 –76	72 –69
	Estradiol-17β	92 –81	83 –74
	Estriol	86 –78	80.5–68
Standard added after autoclaving	Estrone	98.5–75.5	89 –70
	Estradiol-17β	101 –92	90 –88
	Estriol	86 –97	81 –89.5

after which 6 ml. of 65 per cent H_2SO_4 were added, as advised by Bates and Cohen (5). The fluorescence was read with a Coleman electronic photofluorometer, model 12 A, using a Coleman B-2 lamp filter and Coleman PC-9A photocell filter. The sensitivity of the instrument was calibrated with quinine sulfate solution (0.3 mg. per liter in N/10 sulfuric acid) using a Coleman B-1 lamp filter. In each set the fluorescence of standard solutions of estrone, estradiol, estriol and 16-ketoestradiol was determined. A blank was used which had been prepared with the reagents.

Construction of curves: The fluorescence value read in each tube was plotted as ordinate against tube number as abscissa. This method of recording was employed instead of attempting to calculate the readings as micrograms of estrone, as done by Engel (2). Since estradiol and estriol differ in their fluorescence from estrone, false values are obtained if all three estrogens are expressed in equivalence of estrone.

The theoretical distribution curves of the various estrogens in the different systems of solvents were calculated by the method of Williamson and Craig (6).

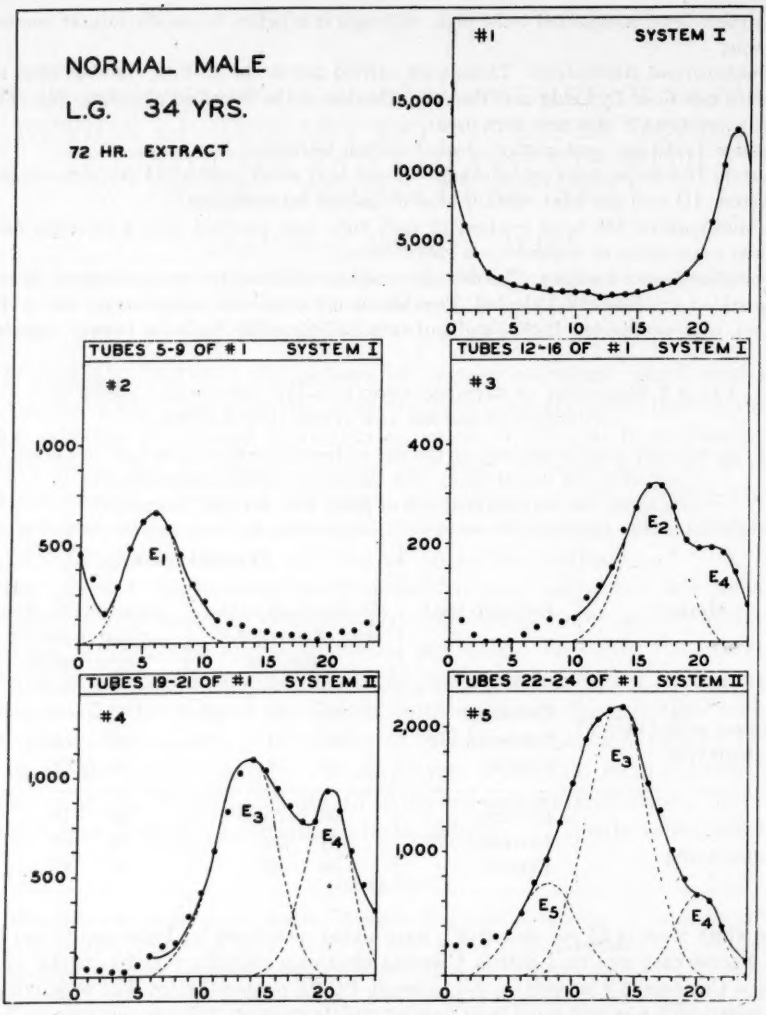

FIG. 1. *Fractionation of urinary estrogens in a normal male.* Curve #1 shows the distribution of the total 72-hour extract. Curves #2, 3, 4 and 5 show the redistribution of indicated tubes from curve #1. (E_1) = estrone; (E_2) = estradiol-17β; (E_3) = estriol; (E_4) = compound with a partition coefficient similar to that of estriol in system I, and of K = 4.5 in system II; (E_5) = compound with a partition coefficient similar to that of estriol in system I, and of K = 0.5 in system II. Broken line is the calculated theoretical curve.

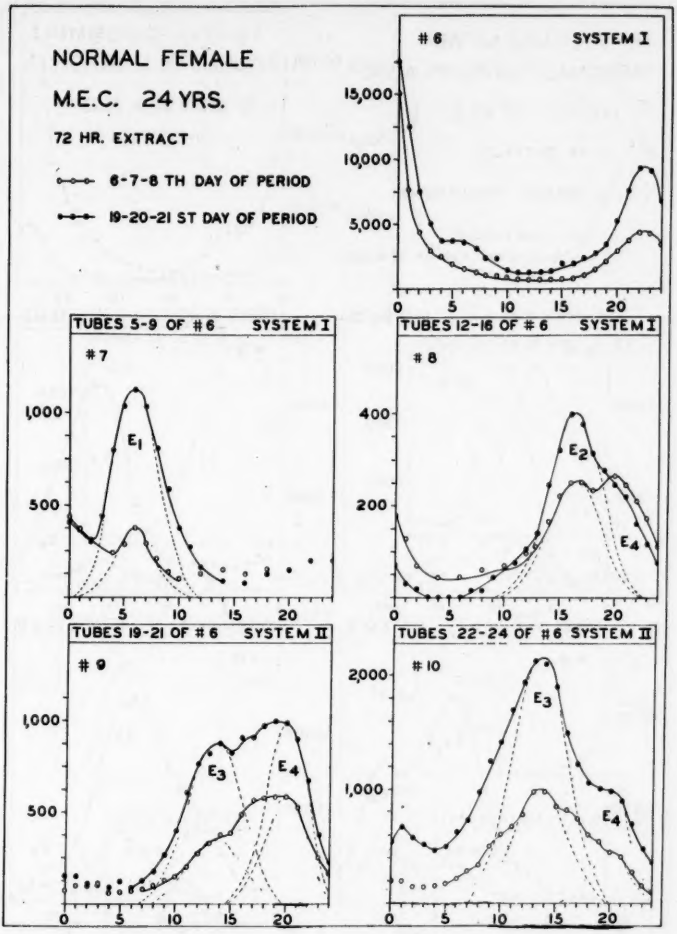

FIG. 2. *Fractionation of urinary estrogens in a normal female.* Curve #6 shows the distribution of the total 72-hour extract at the beginning and at the end of the menstrual cycle. Curves #7, 8, 9 and 10 show the redistribution of indicated tubes from curve #6.

Results with pure compounds and control check of methods: Partition coefficients of estrone, estradiol-17β, estriol and 16-ketoestradiol[2] in the three systems of solvents used in the present work were checked by coun-

[2] Crystalline estrone and estriol were made available through the courtesy of Parke, Davis and Company. Crystalline estradiol-17β was graciously supplied by Ciba Pharmaceutical Products, Inc. We are indebted to Dr. Gregory Pincus and Dr. Max N. Huffman who provided the crystalline 16-ketoestradiol (22).

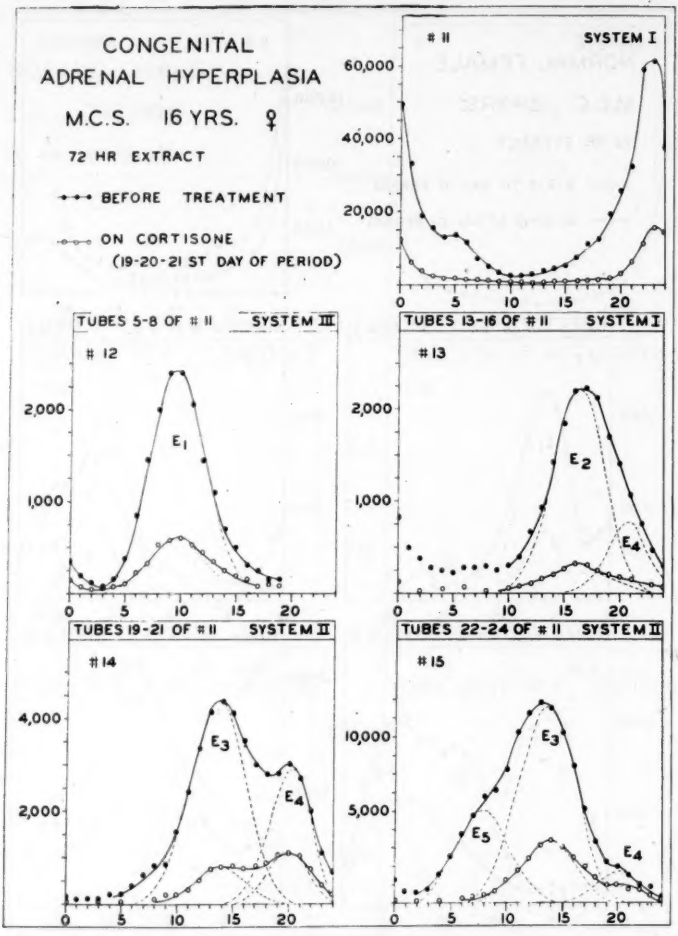

FIG. 3. *Fractionation of urinary estrogens in a female pseudohermaphrodite.* Curve #11 shows the distribution of the total 72-hour extract before and during cortisone therapy. Curves #12, 13, 14 and 15 show the redistribution of indicated tubes from curve #11.

tercurrent distribution and found to be similar to those reported by Engel (2).

The efficiency of the extraction method was tested. Recovery of the three natural estrogens, added after hydrolysis, was 75.5 to 101 per cent (Table 1)—very similar to that found by Engel and colleagues (2). After countercurrent distribution, recovery was lowered (70 to 90 per cent).

Beall (7) suggested that a vigorous hydrolysis, especially at autoclave

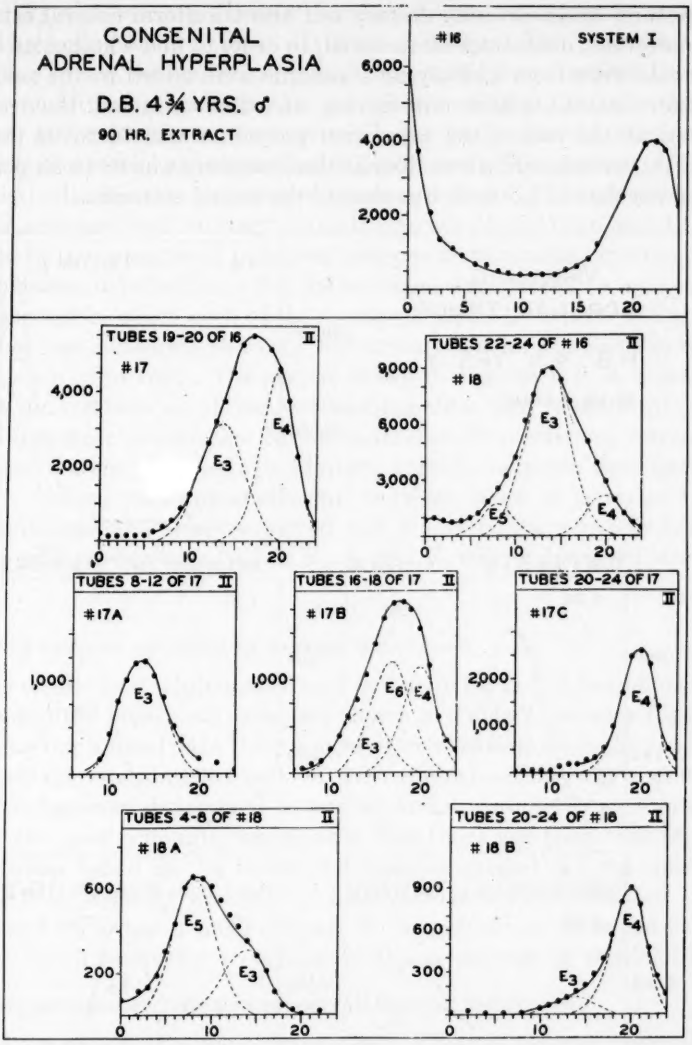

FIG. 4. *Fractionation of urinary estrogens in a case of macrogenitosomia praecox.* Curve #16 shows the distribution of the total 90-hour extract before cortisone therapy. Curve #17 shows the redistribution of tubes 19–20 from curve #16. Curves #17A, 17B and 17C show the redistribution of indicated tubes from curve #17. Curve #18 shows the redistribution of tubes 22–24 from curve #16. Curves #18A and 18B show the redistribution of indicated tubes from curve #18.

temperature, could not only destroy but also transform natural estrogens into fluorescent, nonestrogenic material. In order to find whether such artefacts could arise from hydrolysis, standards were added to the acid solution before instead of after autoclaving, and then extracted. Recovery determined at the end of the extraction procedure was 76 to 92 per cent (Table 1). On countercurrent distribution, recovery was 68 to 83 per cent. The curves showed no peak but that of the tested estrogen.

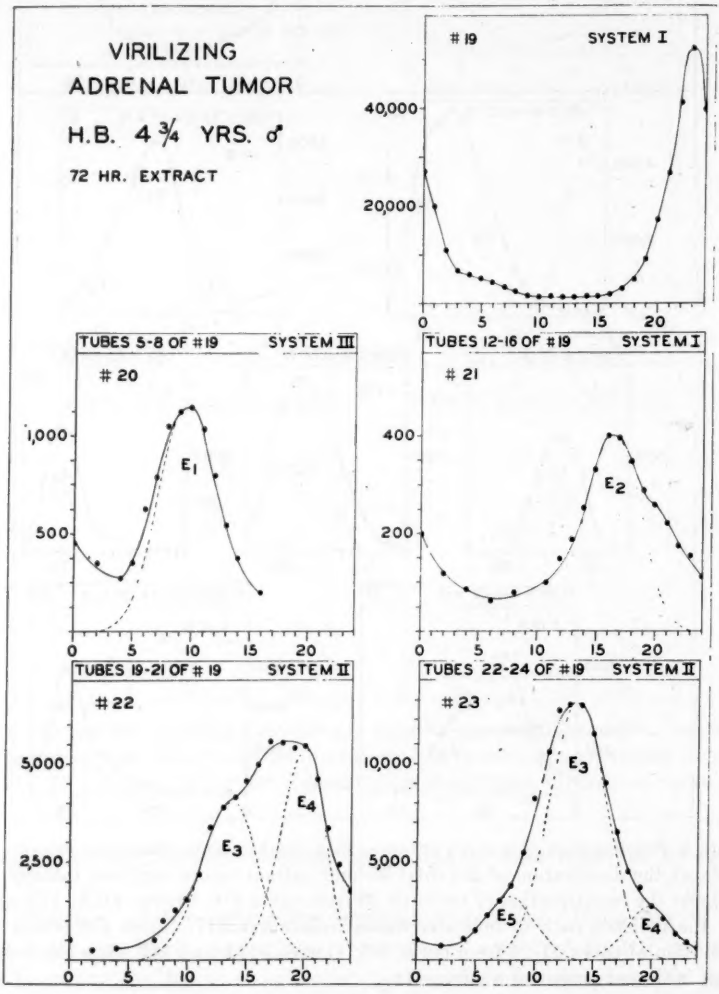

FIG. 5. *Fractionation of urinary estrogens in a case of adrenocortical tumor (Case #2).* Curve #19 shows the distribution of the total 72-hour extract. Curves #20, 21, 22 and 23 show the redistribution of indicated tubes from curve #19.

Results with urinary extracts: For any system of solvents, each of the three estrogens has a definite partition coefficient. The peak tube can be calculated from the equation: $N = nK/K+1$, N being the peak tube, n the number of transfers and K the partition coefficient. In our 24-transfer technic and using the solvent system I, the peak tubes for estrone, estradiol-17β and estriol are respectively 6, 16 and 23. The absence of the characteristic distribution patterns of estrone, estradiol-17β and estriol in the curves obtained with urinary extracts (curves #1, 6, 11, 16 and 19) might be due to the presence of unknown nonspecific fluorescent material. In order to determine whether or not the estrogens were present, a series of tubes was selected in which each of the estrogens might be expected. Each series of tubes was combined and then redistributed, using the same or a different system of solvents. The graphs shown in Figures 1, 2, 3, 4 and 5 illustrate the methods employed for selecting tubes and redistributing them. In all instances distribution curves characteristic of estrone, estradiol-17β and estriol were identified. In addition, certain unknown fluorogenic substances having partition coefficients between those of estradiol-17β and estriol in system I were suspected and separated more or less distinctly. These substances designated as E_4, E_5 and E_6 will be discussed later.

RESULTS

Urinary estrogen excretion in normal individuals

The results in 2 adult males, and in 2 females at the beginning and at the end of the menstrual cycle, are shown in Table 2 (see also Figs. 1 and 2). From our limited data there appeared to be variations in the amounts of estrogen excreted, but the ratio of the various fractions was similar in all cases. Substances distributed as estrone and estradiol-17β were present in about the same amounts, which were four times less than that of estriol. The values found in the males and females studied did not differ very much. In a pregnant woman, the total estrogens were increased 150–300 times and there was a small increase in the percentage of estriol-like compound, accompanied by a decrease in the percentage of estradiol-17β.

Urinary estrogen excretion in congenital adrenal hyperplasia

In 4 patients with adrenal hyperplasia whose cases were previously reported, each fraction was proportionally increased (Table 2 and Fig. 3), so that the ratio of the 3 fractions did not differ essentially from that in normal individuals. Cortisone therapy, in doses which suppressed urinary 17-ketosteroid excretion (8, 9), decreased each estrogen fraction so that the ratio remained the same.

Urinary estrogen excretion in adrenocortical tumor

In 2 cases of adrenocortical tumor, the total increase of urinary estro-

TABLE 2. FRACTIONATION BY COUNTERCURRENT DISTRIBUTION OF URINARY ESTROGENS IN NORMAL INDIVIDUALS AND PATIENTS WITH HYPERADRENOCORTICISM.

(The case numbers recorded in this table are the same as in the previous report (13). Patient D.P. (# 4) received some cortisone between the first and second determination: this transitory therapy may account for the decrease in estrogens. The values in brackets are the percentage of each fraction.)

	Patient	Sex	Age (yrs.)	Condition	Urinary estrogens γ/24 hrs. of compound which distributes as: Estrone	Estradiol-17β	Estriol	Total	Urinary 17-KS* (mg./24 hrs.)	FSH† (m.u./24 hrs.)
Controls	C.M.	M	28		5 (17%)	4.5 (15%)	20 (68%)	29.5	10.5	
	L.G.	M	34		6 (12%)	7 (14%)	36 (74%)	49	16.5	+ for 6.6 and 13.2; − for 26.4 and over
	M.E.C.	F	24	6–7–8th days of cycle	3 (13%)	5.5 (22%)	15.5 (65%)	24	8.5	
				19–20–21st days of cycle	10 (18%)	10 (18%)	35 (64%)	55	9.5	+ for 6.6; − for 13.2 and over
	L.D.	F	26	6–7–8th days of cycle	6 (16%)	7 (17%)	27 (67%)	40	11.0	
	J.R.	F	20	6th mo. of pregnancy	700 (11%)	205 (3%)	5,650 (86%)	6,555	8.8	
				7th mo. of pregnancy	1,120 (15%)	270 (4%)	6,200 (81%)	7,590	11.5	
Congenital adrenal hyperplasia	#4 D.P.	F	49	No treatment	25 (7%)	43 (11%)	313 (82%)	381	68.0	
				After ovariectomy	13.5 (6%)	25 (12%)	170 (82%)	208.5	30.8	+ from 6.6 to 192; − for 384 and over
				On cortisone 2 mos.	4 (16%)	3.5 (14%)	18 (70%)	25.5	5.5	+ for 6.6 to 96; − for 192 and over
	#6 M.C.S.	F	16½	No treatment	27.5 (10%)	46 (16%)	210 (74%)	283.5	59.1	− from 6.6 to 52.8
				On cortisone 18 mos.	5.5 (10%)	6 (22%)	42 (78%)	53.5	3.0	+ from 6.6 to 26.4; − for 52.8
	#14 D.B.	M	9	No treatment	18.5 (10%)	38.5 (22%)	120 (68%)	177	35.5	− for 6.6 and over
				On cortisone 6 mos.	3.0 (15%)	3.5 (17%)	14 (68%)	20.5	3.6	− for 6.6 and over
	#16 W.D.	M	3½	No treatment	11 (10%)	24 (23%)	70 (67%)	105	25.2	− for 6.6 and over
				On cortisone 7 mos.	4 (18%)	5 (23%)	13 (59%)	22	1.7	− for 6.6 and over
Adrenal tumor	#2 H.B.	M	4¼	No treatment	13 (6%)	10 (4%)	196 (90%)	219	194	− for 6.6 and over
	#1 E.H.	F	35	No treatment	18 (8.5%)	16 (7.5%)	180 (84%)	214	84.5	

* The 17-ketosteroids were determined by the method of Callow *et al.* (22), modified by Jensen (23).

† The FSH was measured by the method of Klinefelter *et al.* (24).

gens was largely due to the increase of the estriol fraction. The estriol fraction represented 84 per cent of the total in Case 1 and 90 per cent in Case 2 (see Table 2 and Fig. 5).

Presence of other fluorogenic compounds in estrogen extracts

In each case, the fluorescence of the 25 tubes on the first distribution in system I was added, the total representing the total fluorescence of this urinary extract. The specific fluorescence caused by the estrone, estradiol-17β and estriol of the urinary extract was from 19 to 32 per cent of the total, in both normal males and females. In a pregnant female the specific fluorescence represented a much larger amount of the total—73 to 74 per cent. In congenital adrenal hyperplasia it was 26.5 to 41 per cent before

TABLE 3. PERCENTAGE OF SPECIFIC FLUORESCENCE AND OF NONSPECIFIC FLUORESCENT MATERIAL

(E_4) = Substance with a partition coefficient similar to that of estriol in system I, and of K = 4.5 in system II.

(E_5) = Substance with a partition coefficient similar to that of estriol in system I, and of K = 0.5 in system II.

	Patient	Sex	Age (yrs.)	Condition	Per cent of total fluorescence			
					As estrone +estriol +estradiol-17 β	In tubes 0–3 of first fractionation in system I	As substance (E_4)	As substance (E_4)
Controls	C.M.	M	28		21	48	3.5	7
	L.G.	M	34		32	24.5	6	9
	M.E.C.	F	24	6-7-8th days of cycle	19	42.5	3	7.5
				19-20-21st days of cycle	28	37	2.9	10
	L.D.	F	26	6-7-8th days of cycle	24	34	3.8	8.5
	J.R.	F	20	6th mo. of pregnancy	73	3.5	0.9	10
				7th mo. of pregnancy	74	2.5	0.8	14
Congenital adrenal hyperplasia	#4 D.P.	F	49	No treatment	41	17.5		13.5
				After ovariectomy	33	15	7.3	10
				On cortisone 2 mos.	19	35	4.7	9
	#6 M.C.S.	F	16½	No treatment	32	25	7.2	6.5
				On cortisone 18 mos.	36	26	2.5	12.5
	#14 D.B.	M	9	No treatment	26.5	29	3	11.5
				On cortisone 6 mos.	20	38	4.4	11
	#16 W.D.	M	3½	No treatment	28	27	4.1	13.5
				On cortisone 7 mos.	28	37	5	12
Adrenal tumor	#2 H.B.	M	4½	No treatment	33	29	3.7	15
	#1 E.H.	F	35	No treatment	29	31	5.1	10

† The FSH was measured by the method of Klinefelter *et al.* (24).

treatment and 19 to 36 per cent during cortisone therapy. It averaged the same amount in adrenocortical tumor of the virilizing type.

A great part of the nonspecific fluorescence was due to material having a very low partition coefficient and, therefore, appearing in the first few tubes in system I. The fluorescence of tubes 0 to 3 in each case was added and the percentage of the total fluorescence that it represented was calculated. The results are shown in Table 3. Except in the case of pregnancy, it represented an average of 30 per cent of the total fluorescence (from 15 to 48 per cent).

A smaller amount of nonspecific fluorescence was due to compounds having a partition coefficient in system I similar to that of estriol. In system II, it was possible to distinguish two compounds different from estriol having partition coefficients of 0.5 and 4.5 (Fig. 4, curves #18A and 18B). No significant difference was found in the percentage of these two compounds in normal controls, in pregnancy or in hyperadrenocorticism (Table 3).

DISCUSSION

Several methods for the fractionation of estrogens have been recommended, such as alumina column (10–11), paper chromatography, and Girard's reagent for separation of estrone. Engel (12) pointed out the advantages of countercurrent distribution. However, a single distribution in one system of solvents did not permit the separation of the natural estrogens in certain cases, for example in males (2). In the present study we found it necessary to redistribute certain tubes of the initial fractionation. In this way it was possible to show that a part of the fluorescence of male urinary extract was due to substances resembling the three natural estrogens (Fig. 1) and that the percentage of nonspecific fluorescence was not larger than in normal female urinary extracts. It was also possible to demonstrate compounds distributing as estriol and estradiol-17β in the urine of normal females at the beginning of the menstrual cycle (Fig.2), contrary to the data reported by Engel (12). This may be due to differences in the method of purification of the extracts.

In adrenal hyperplasia, there was an increase of urinary estrogens, as shown previously (13). Cortisone therapy reduced the estrogens as well as the 17-ketosteroids to a normal level. In cases of adrenal tumor there was also an increase of urinary estrogens, but it was demonstrated in earlier studies that the administration of cortisone did not decrease either the estrogens or the elevated 17-ketosteroids (13–14). It was suggested that this difference in the action of cortisone might serve as a test to distinguish between adrenal hyperplasia and adrenal tumor.

In the control group, except in pregnancy, there was an almost constant ratio between the three urinary fluorogenic fractions. This was true also in

congenital adrenal hyperplasia, before and during cortisone therapy. During pregnancy and in cases of adrenocortical tumor there appeared to be an increase of the estriol-like fraction, but our experience was too limited to permit a definite conclusion on this point. Estrogens can be secreted by ovaries, adrenal glands, placenta and the testes (15). Since the urinary estrogens represent the end point of estrogen metabolism, it is impossible to draw conclusions concerning the nature of the estrogen actually secreted by the endocrine glands. Whatever the secreting organ may be, and whatever the hormone secreted, at the end the ratio of the urinary estrogens appear to be the same.

Except during pregnancy, only 30 per cent of the total fluorescence could be accounted for by the quantities of fractions resembling estrone, estradiol-17β and estriol. Although the nonspecific fluorescence may have no relation to the estrogenic hormones, the possibility exists that it is due to compounds which are hormones themselves, or are intermediary metabolites of the estrogens. The reciprocal transformation of estrone into estradiol-17β has been established (16). These two estrogens can also be transformed irreversibly into estriol. Marrian (17) proposed several possible intermediaries for the transformation of estrone and estradiol-17β into estriol. One of them, 16-ketoestradiol-17β, has a partition coefficient in system I and II (18) quite similar to one of the compounds, designated as E_4, that we found in all urinary extracts. We were even able to separate this compound E_4 from estriol (Fig. 4, curve #18B). However, the fact that when we subjected crystalline 16-ketoestradiol-17β to the method of hydrolysis and extraction employed in this work, we were no longer able to recover it, suggested that E_4 was not this compound. Slaunwhite (19) also noted the presence of a similar compound, but did not isolate it. Another compound, designated as E_5, was also separated (Fig. 4, curve #18A) but in much smaller amounts than E_4. It was suspected that still another compound (E_6) might be present (Fig. 4, curve #17B). Zondek and Finkelstein (20) found that there was a ratio 5:1 between the estrone fraction measured fluorometrically and that measured biologically; they suggested that it was due to some estrogen metabolite of low biologic activity which fluoresced as much as estrone. Lieberman and colleagues (26) suggested the presence of end products of the *in vitro* reaction of liver on 17β-estradiol—products which are different from estrone, estriol or conjugated estrogens. It would be important to know the chemical and biologic properties of these various substances.

CONCLUSIONS

1. Countercurrent distribution followed by fluorometric measurement of urinary estrogen extracts was found to be a satisfactory method of

fractionating the natural estrogens and separating them from nonspecific fluorescent material.

2. There was a rather constant ratio between fractions resembling estrone, estradiol-17β and estriol in 4 normal subjects (male and female) and in 4 cases of congenital adrenal hyperplasia before and during cortisone therapy. There was some increase of the estriol fraction in 1 pregnant female and in 2 patients with adrenocortical tumor of the virilizing type. Only 19–41 per cent of the total fluorescence was presumably due to the three estrogens.

3. From the nonspecific fluorescent material it was possible to separate two other substances. The substance designated as E_4 represented 7–15 per cent of the total fluorescence; the substance designated as E_5 represented 2.5 to 7.3 per cent. Their chemical composition was not determined.

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COMPARATIVE CLINICAL EVALUATION OF ESTROGENIC PREPARATIONS BY THE MENOPAUSAL AND AMENORRHEAL INDICES*†

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ESTROGENS for clinical use are usually standardized either by animal assay or by unit weight of crystalline material. Although such methods yield some information as to the expected comparative efficacy of these preparations in man they are inadequate for exact dosage relationships in clinical medicine. Hence, it is desirable that the relative activity of estrogens be based upon human "assay" rather than biologic or chemical standardization procedures.

The investigation of the clinical efficacy of different hormonal preparations, however, presents many difficulties and must, of necessity, entail large series of subjects to offset the inroads of biologic variation. Unfortunately, in the clinical evaluation of the effectiveness of steroid estrogens or their stilbene confreres, greater variability of response and less predictability of results occur than in animal assay procedures. In the latter assay methods one can adhere to a rigid selection of an adequate number of properly standardized subjects. The hormonal deficiency state produced in the laboratory animal according to the appropriate assay technique also provides an adequate base level from which one can establish the degree of response of the animal to the administered endocrine preparation. Such hypoglandular states cannot be induced at will clinically, and only patients exhibiting endocrine insufficiencies can be employed in the human evaluation of the different estrogenic hormones. Such studies are greatly facilitated if the hormone to be tested produces certain measurable hematologic, chemical, or physiologic alterations in bodily functions—changes which are proportional to the activity of the prepara-

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tion and which can be measured quantitatively. On the other hand, the problem of "human assay" is magnified many fold when one must depend upon correction of subjective complaints in judging the clinical efficacy of preparations.

The comparative effectiveness of an estrogen can, nevertheless, be determined clinically in adequately designed studies in hypogonadal states in menopausal females or in women with primary or secondary amenorrhea (1, 2). Although the criteria for judging the activity of estrogens are more objective in the amenorrheal patient, they tend to approach nebulous proportions with respect to the menopausal patient. The critical evaluation of the activity of both natural and synthetic estrogens in the menopausal patient has been encompassed by the purely subjective criteria employed in such studies and, indeed, has been influenced by the inclination of the investigator himself. When data have been obtained, it has been difficult to analyze the material adequately to permit exact comparison of one preparation with another.

The ability of estrogens to induce withdrawal bleeding, on the other hand, may be measured objectively by noting the approximate extent and duration of estrogen withdrawal bleeding (2). Estrogen withdrawal bleeding, however, may be misleading in slow-acting estrogens, *i.e.*, so-called "pro-estrogens," since there is no sharp drop in estrogen level, and estrogen withdrawal bleeding may be delayed in onset or even absent unless desquamation may be induced by the subsequent addition of progesterone.

In order to bring about a more objective means of evaluating different estrogenic compounds in menopausal and amenorrheal patients, a new system of judging and estimating the clinical response of such patients to estrogens has been devised—the menopausal index and amenorrheal index (3), respectively. This is based upon classifying data from these two groups of patients by means of numerical conversion factors. These indices provide objective clinical methods for the evaluation of the efficacy of different estrogenic compounds. They also permit a statistical compilation of our data once collected; this avoids basing our clinical impression of the different preparations upon "the shifting sands of empiricism." Since the menopausal and amenorrheal indices are based upon different components and end points, they are discussed separately. The comparative effects of a large series of estrogens upon these two indices are presented.

METHODS AND RESULTS

The menopausal index

The menopausal index, which will be considered first, represents the sum of the numerical conversion factor of the eleven most common meno-

pausal complaints. These subjective findings are converted to a numerical figure based upon the severity and extent of these complaints. The sum of these individual numbers serves as a guide to the severity of the menopausal symptoms and is designated as the menopausal index.

The symptoms used in devising this index are as follows: vasomotor complaints, paresthesia (numbness, tingling sensation, and temperature changes), insomnia, nervousness, melancholia, vertigo, weakness or fatigue, arthralgia and myalgia, headaches, palpitation, and formication. The first four of these symptoms are most prevalent and of greater consequence and are, therefore, given a so-called weighted factor to lend additional emphasis to them in formulating the menopausal index. Inasmuch as the vasomotor symptoms, although a comparatively late manifestation of the menopause, are by far the most common, they are given the greatest degree of weight, namely, a factor of 4. We feel this is justified, since hot flashes are usually the major consideration responsible for the patient's presenting herself to the physician. Thus, whatever the numerical conversions of the severity of the vasomotor symptoms are, they are arbitrarily multiplied by four. Paresthesia, insomnia, and nervousness, on the other hand, although outranking the remaining symptoms, are inferior in importance to the vasomotor complaints and are, therefore, given a factor of 2. The remaining symptoms have a factor of 1. The numerical conversion employed in obtaining the menopausal index is secured by multiplying the foregoing factors by the numerical equivalent of the severity noted for the symptoms. The numerical severity of the symptoms is based upon the following system: If the patient has no complaints referable to a particular symptom, the symbol "O" is placed on the chart for that date alongside of the symptom and the numerical conversion figure is likewise O. However, if the patient complains of slight symptoms, the symbol "S" is placed on the chart and the arithmetic equivalent of "S" is 1. If the patient complains of moderate symptoms, the letter "M" is placed on the chart and the numerical conversion for this degree of severity is 2. Finally, if the patient complains of marked symptoms, then a "+" is placed on the chart and the conversion factor is 3. An example of the theoretical menopausal index is outlined in the accompanying Figure 1; although purely arbitrary, it is self-explanatory. It is to be noted that whereas the numerical conversion factor is the final figure in the seven symptoms listed in the lower part of the graph, the factors for the top four symptoms are first multiplied by the weighted figure before the final conversion number is obtained. The sum of these figures is then recorded and is considered as the menopausal index.

Besides obtaining information permitting formulation of the menopausal index, other data are also obtained from the climacteric patient which

MENOPAUSAL INDEX

SYMPTOMS	FACTOR	SEVERITY	NUMERICAL CONVERSION
1. VASOMOTOR	4	+ = 3	12
2. PARESTHESIA	2	M = 2	4
3. INSOMNIA	2	M = 2	4
4. NERVOUSNESS	2	S = 1	2
5. MELANCHOLIA	1	0 = 0	0
6. VERTIGO	1	0 = 0	0
7. WEAKNESS (FATIGUE)	1	M = 2	2
8. ARTHRALGIA AND MYALGIA	1	S = 1	1
9. HEADACHES	1	+ = 3	3
10. PALPITATION	1	M = 2	2
11. FORMICATION	1	S = 1	1
MENOPAUSAL INDEX (SUM)			31

CODE

0 - NONE = 0 M - MODERATE = 2

S - SLIGHT = 1 + - MARKED = 3

FIGURE 1

have been designated as the clinical improvement. Clinical improvement may range from 0 to 4+ and is dependent upon how the patient herself evaluates her response as a whole to the administered preparation. A clinical improvement of 4+ is noted in a patient who feels perfectly well and judges that all symptoms have reverted more or less to essentially a normal state. A 3+ classification indicates a marked improvement, but some remaining complaints. A 2+ evaluation refers only to moderate improvement, 1+ to slight improvement, and 0 implies that there is no clinical improvement as far as the patient can determine.

In addition to these two factors, *i.e.*, the menopausal index and the clinical improvement criteria, vaginal smears were also obtained on all patients upon their return to the endocrine clinic and were graded from normal, or normal with increased cornification, down to a 4+ estrogenic deficiency. A 1+ smear is indicative of mild deficiency; 2+, moderate deficiency; 3+, marked deficiency; and 4+, very marked estrogenic deficiency. The actual extent of the deficiency is correlated with the presence of basal cells. A 4+ smear is one characterized by a predominance of basal cells, whereas normal smears are noted for their absence of basal cells and a predominance of cornified cells, or equal parts of cornified and intermediate cells. The smear classified as normal with increased cornification is one consisting predominantly of cornified cells with only a few intermediate cells. Smears labelled 2+ and 3+ indicate grades in between the

1+ and 4+ classification, and are also evaluated upon the basis of the relative number of basal, cornified and intermediate cells present in the vaginal spread.

It is also important to note that in the major portion of this study a blind testing program had been instituted. The system devised is one based upon the fact that the interrogator, who has invariably been the same investigator throughout this study, did not have any knowledge of the type of preparation employed. By adhering to the blind testing procedure in a clinical evaluating study, it must be agreed that observations so obtained are truly objective and cannot be influenced by the subjective concept the physician may have of the effectiveness of the drug which the patient has taken (4). In addition, the clinical studies were controlled to a greater extent by liberal use of placebos and other nonestrogenic compounds. The drugs were administered to the patient by code numbers so that not only was the interrogator unaware of the preparation used, but the patient also had no means of identifying the compound, thus filling the requirements of a double blind testing technique.

The preparations used in this study included a wide group of estrogens of both the synthetic and so-called steroidal groups. In the synthetic group, we used stilbestrol, hexestrol, dinestrol, and a more recent compound, TACE, considered as a pro-estrogen. Of the steroidal estrogens, estradiol in the form of free estradiol, ethinyl estradiol, estradiol linguets, and a combination of free and ethinyl estradiol with and without methyltestosterone, were also evaluated according to the prescribed scheme. In addition to these, the following compounds were employed: conjugated estrogens (equine), equine estrogenic factors, piperazine estrone sulfate, potassium estrone sulfate, crystalline estrone sulfate, and crystalline estrone sulfate—enteric coated. The latter four of these compounds are crystalline synthetic compounds and were available in purified form. The equine estrogenic factors employed were standardized in equivalents of international units of estrone, whereas conjugated estrogens (equine) were standardized in equivalents of sodium estrone sulfate. Vitamin E preparations, phenobarbital, and placebos were intermixed with the above estrogenic compounds, not according to any described procedure, but in such a manner that the interrogator would not be aware of the medication being used.

The patients were usually seen at four-week to six-week intervals, at which times they were interrogated concerning their symptoms and recordings were made on the menopausal history chart. At the time that the patient was seen for questioning, a vaginal smear was taken prior to the patient-physician interview.

The doses of the various preparations used were uniform for each individual drug and are listed in Table 1. TACE was administered in a dose

TABLE 1. THE EFFECT OF ESTROGENIC AND NONESTROGENIC COMPOUNDS UPON MENOPAUSAL SYMPTOMS

Preparation	Dose	No. of patients	Response to Therapy					
			POOR		MODERATE		EXCELLENT	
			No. of patients	Per cent	No. of patients	Per cent	No. of patients	Per cent
Conjugated estrogens (equine—Premarin)	1.25 mg., daily	156	11	7.0	41	26.3	104	66.7
Piperazine estrone sulfate	1.5 mg., daily	55	6	10.9	8	32.7	31	56.4
Potassium estrone sulfate	1.25 mg., daily	76	9	11.8	25	32.9	42	55.3
Estrone sulfate (crystalline)	1.25 mg., daily	80	17	21.2	21	26.3	42	52.5
Estrone sulfate (enteric coated)	1.25 mg., daily	81	15	18.4	27	33.3	39	48.3
Ethinyl estradiol (Estinyl)	0.05 mg., daily	88	7	8.0	23	26.0	58	66.0
Estradiol	2.0 mg., daily	33	4	12.1	11	33.4	18	54.5
Estradiol linguets	0.25 mg., daily	28	3	10.8	11	39.2	14	50.0
Equine estrogenic factors	10,000 I.U., daily	79	21	26.6	24	30.4	34	43.0
TACE	12 mg., daily or 24 mg., 3×wk.	85	13	15.2	22	25.8	50	59.0
Stilbestrol	0.1 mg., 3×dy.	47	19	40.4	14	29.8	14	29.8
Hexestrol	3 mg., daily	42	15	35.7	12	28.6	15	35.7
Dinestrol	0.5 mg., daily	71	23	32.4	25	35.2	23	32.4
Estradiol Ethinyl estradiol	0.4 mg. 0.04 mg. } daily	66	6	9.1	22	33.3	38	57.6
Estradiol Ethinyl estradiol Methyltestosterone	0.4 mg. 0.04 mg. 2.5 mg. } daily	34	4	11.8	6	17.7	24	70.5
Vitamin E	50–100 mg., daily	69	53	76.75	5	7.25	11	16.0
Phenobarbital	15 mg., 3×dy.	89	51	57.3	17	19.2	21	23.5
Placebo	1 tab., daily	248	165	66.6	32	12.8	51	20.6

of either 12 mg. per day or 24 mg. every second day. A more complete evaluation of this latter compound will be the subject of a subsequent report. Vitamin E was administered in doses of 50–100 mg. in capsule or tablet form. Phenobarbital was given in 15-mg. doses and stilbestrol in 0.1 mg. doses three times a day. All other medications were taken once a day.

Analysis of our data presented in Table 1 and Figure 2 are based upon consideration of both the menopausal index and clinical improvement. From these data the effectiveness of therapy was judged as poor, moderate, or producing excellent results. It may be seen that of all preparations used, ethinyl estradiol, alone or in combination with free estradiol and conjugated estrogens (equine), on the average yielded superior results in controlling the menopausal symptoms. The use of these preparations adequately controlled the symptoms of the climacteric patient and produced the smallest incidences of failures. The crystalline estrone sulfate derivatives, contrary to some reports, did not compare in effectiveness to conjugated estrogens (equine) on an equivalent dose basis (5–7).

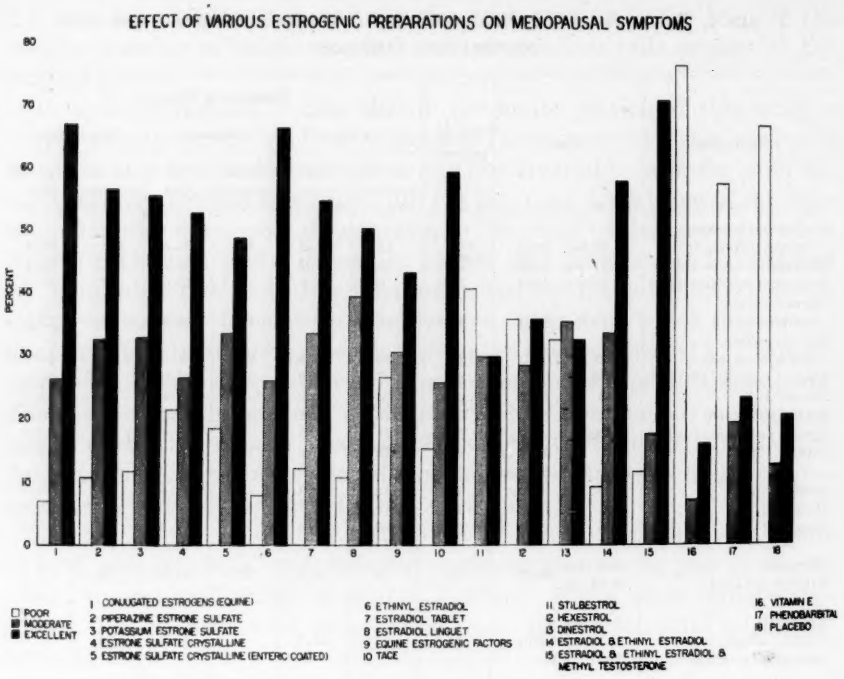

FIGURE 2

No significant side effects were noted for any of these preparations. Stilbestrol produced the highest incidence of nausea and had to be discontinued in 20 per cent of the patients because of gastrointestinal disturbances. Conjugated estrogens (equine) induced skin eruption in 2 cases. Both of these patients failed to show skin sensitivity with the crystalline synthetic material. Withdrawal bleeding was rarely observed with any of the preparations employed, with the exception of the combination of free and ethinyl estradiol. Withdrawal bleeding was noted in 18 per cent of those patients who received the combined free and ethinyl estradiol.

TACE, in the doses used, proved to be an effective compound in controlling menopausal symptoms and failed to show significant undesirable side effects. However, as seen in Table 1, no preparation, was entirely free from failure and no preparation gave 100 per cent excellent results. It is to be noted from the data in Table 1 and Figure 2 that vitamin E is no more effective than a placebo. This is in contrast to earlier reports on the therapeutic effectiveness of vitamin E in the climacteric (8–16). Further evaluation of vitamin E and comparison of the estrone sulfates, crystalline

and noncrystalline equivalent compounds, will be discussed in subsequent reports. The addition of methyltestosterone to the estradiol combination enhanced the clinical efficacy of the estrogenic compound, substantiating other reports on the synergism between estrogens and androgens in the treatment of the female climacteric (17–20).

Vaginal smear data did not reveal a definite correlation between the clinical efficacy of the preparation and the degree of cornification it induced (1, 21). However, it must be noted that not all the climacteric patients showed manifestations of estrogenic deficiency in smears taken before the onset of endocrine therapy. Although the degree of vaginal cornification varied in different patients with the various estrogens used, increased cellular maturation or vaginal cornification was the rule in the majority of the patients during estrogenic therapy.

The amenorrheal index

A further means of evaluation of the effectiveness of estrogenic compounds was undertaken by the use of another comparative index termed the amenorrheal index. This criterion, as in the case of the menopausal index, represents a numerical conversion factor, but the amenorrheal index measures the effectiveness of an estrogenic compound followed by a constant dose of progesterone in producing withdrawal bleeding in patients with primary or secondary amenorrhea (3). The patients used in this study were those in whom menstrual periods had not occurred for at least six months and in whom progesterone as a rule, when given alone, failed to induce a withdrawal menstrual flow. By the addition of progesterone, we were able to obtain a significant endometrial desquamation or menstrual flow in patients adequately primed with long-acting or short-acting estrogens. The amenorrheal index was obtained under as standard conditions as possible. Progesterone by its desquamative action on an estrogenic primed endometrium automatically induces a prompt menstrual flow. Withdrawal bleeding following estrogen alone may at times be slow and long continued, thereby defeating a comparative analysis of different estrogens (2). The estrogen to be tested was administered daily for three weeks in doses twice those usually employed in the menopausal patient. At the end of the three weeks, the patient received a single intramuscular injection of 100 mg. of progesterone in oil.[1] No further therapy was given and the time of withdrawal bleeding was observed. Notes on the duration and extent of withdrawal bleeding were also recorded. From these data, the amenorrheal index was obtained as follows: The time required for

[1] Kindly supplied as Proluton (50 mg. per cc.) by Dr. Norman Heminway of the Schering Corporation.

onset of bleeding in days, calculated from the day of injection of progesterone to the first day of bleeding, was divided into the duration of withdrawal menstrual bleeding in days. The quotient obtained was then multiplied by an estimate of the extent of bleeding, varying from 0 to 5+. Thus, 1+ bleeding referred to staining; 2+ described a mild to moderate flow; 3+ was considered as a diminished flow, but more than moderate; 4+ designated a normal menstrual flow; and in some cases we have referred to withdrawal bleeding as being 5+ (characterized by flooding or hypermenorrhea). The numerical figure obtained by multiplying the extent of bleeding by the quotient of the duration of bleeding, divided by the time of onset of bleeding in days after the administration of a single 100-mg. dose of progesterone to a patient who had previously received a three-week course of estrogens, was designated as the amenorrheal index. It is graphically represented in Figure 3. The index is based upon the prem-

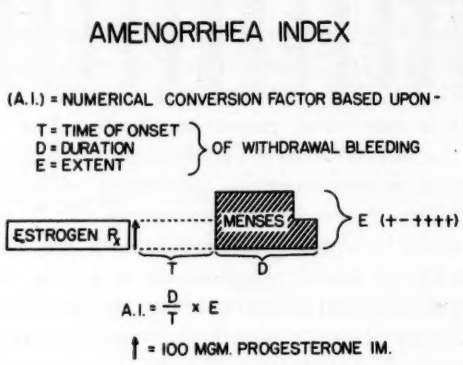

FIGURE 3

ise that the more effective the priming of the endometrium by an estrogenic preparation, the more rapidly will withdrawal bleeding occur and the greater its extent and duration will be after a single injection of progesterone.

A summation of our data on the amenorrheal index is presented in Figure 4 and Table 2. The dose of estrogen employed is equal to twice the menopausal dose noted in Table 1. Here we see, as in the menopausal index, that ethinyl estradiol, alone or in combination with free estradiol, and conjugated estrogens (equine) were the most effective preparations. It may also be noted that the crystalline estrone sulfate compounds were not as effective in priming the uterus for satisfactory menstrual flow as were the conjugated estrogens (equine) standardized in equivalents of sodium estrone sulfate. At the same time, it is evident that piperazine

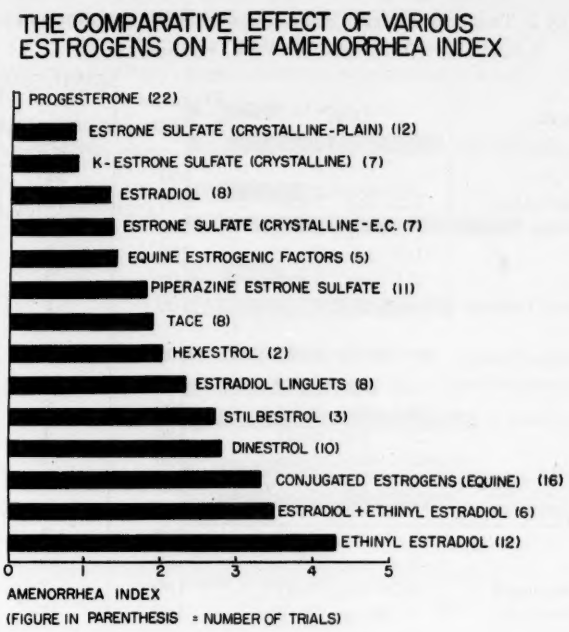

FIGURE 4

estrone sulfate was only slightly better than the crystalline estrone sulfate, but still inferior to the conjugated estrogens (equine). In the middle group of compounds, intermediately effective between conjugated estrogens (equine) and ethinyl estradiol on one hand and crystalline estrone sulfate on the other, are the preparations—TACE, hexestrol, estradiol linguets, stilbestrol, and dinestrol—that were uniformly effective but less active than conjugated estrogens (equine) or ethinyl estradiol in priming the uterus for withdrawal bleeding after a single injection of progesterone. In order to check the quantitative accuracy and validity of the amenorrheal index and our methods, a small series of patients received several doses of the same preparation. This was done with conjugated estrogens (equine), estradiol, ethinyl estradiol, TACE, and ethinyl estradiol plus free estradiol. It must be noted that when the higher doses were employed, a higher amenorrheal index was obtained (Fig. 5). Use of one-half the original dose resulted in an amenorrheal index approximately one-half as great. Combining all the data of the amenorrheal indices on the two dose levels, we see that 3.65 was obtained in 38 trials as compared with 1.6 in 17 trials for the one-half dose level (Table 3). These data tend to support the assumption previously made, that the more active the preparation, the more prompt will

TABLE 2. THE COMPARATIVE EFFECTS OF VARIOUS ESTROGENS UPON THE AMENORRHEAL INDEX

Estrogen	Dose*	Progesterone† (mg.)	No. of patients	Amenorrheal index
—	—	100	22	0.027
Estrone sulfate (plain)	1.25 mg., 2×dy.	100	12	.84
Potassium estrone sulfate	1.25 mg., 2×dy.	100	7	.9
Estradiol	1.0 mg., 2×dy.	100	8	1.3
	2.0 mg., 2×dy.	100	2	3.5
Estrone sulfate (enteric coated)	1.25 mg., 2×dy.	100	7	1.34
Equine estrogenic factors	10,000 I.U., 2×dy.	100	5	1.37
Piperazine estrone sulfate	1.5 mg., 2×dy.	100	11	1.8
TACE	25 mg., 3×wk.	100	8	1.85
	12 mg., daily	100	4	2.5
	12 mg., 2×dy.	100	2	3.75
Hexestrol	3.0 mg., 2×dy.	100	2	2.0
Estradiol linquets	0.25 mg., 2×dy.	100	8	2.3
Stilbestrol	0.2 mg., 3×dy.	100	3	2.7
Dinestrol	0.5 mg., 2×dy.	100	10	2.8
Conjugated estrogens	1.25 mg., daily	100	2	1.2
(equine—Premarin)	1.25 mg., 2×dy.	100	16	3.3
Estradiol Ethinyl estradiol (Estinyl)	0.4 gm. 0.04 mg. } daily	100	1	1.8
	0.4 mg. 0.04 mg. } 2×dy.	100	6	3.5
Ethinyl estradiol	0.05 mg., daily	100	2	1.5
	0.05 mg., 2×dy.	100	12	4.3

* Administered for three weeks.

† Administered as a single injection i.m. in 2 cc. of vegetable oil.

menstrual bleeding follow administration of progesterone, the greater will be the duration and extent of flow, and therefore the higher will be the amenorrheal index.

TABLE 3. COMBINED AMENORRHEAL INDEX OF PATIENTS RECEIVING TWO DIFFERENT DOSAGES OF VARIOUS ESTROGENS

Patient group	Number of trials	Dose	Average amenorrheal index
A*	17	½	1.6
B	38	1	3.65

* Patients in group A received half the dose of the various estrogens which were taken by Patients in group B.

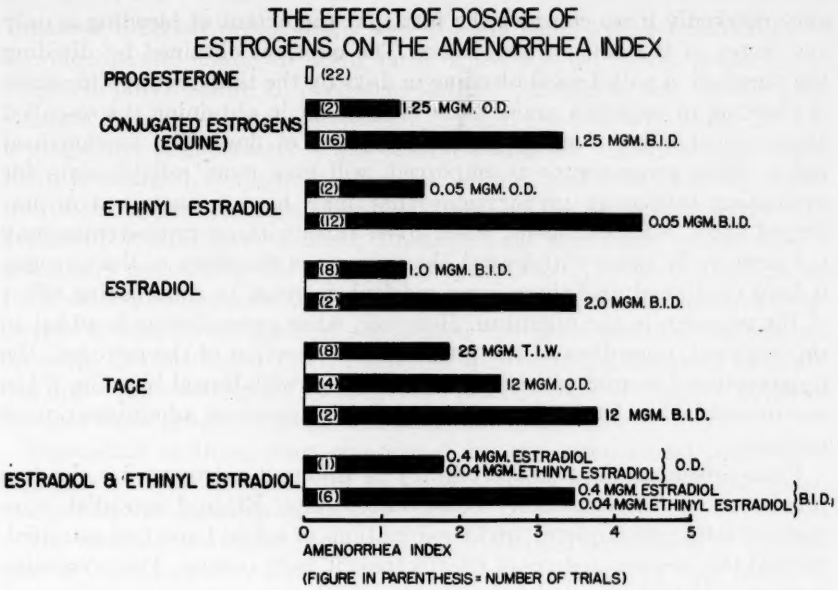
THE EFFECT OF DOSAGE OF
ESTROGENS ON THE AMENORRHEA INDEX
PROGESTERONE
(22)
CONJUGATED ESTROGENS (EQUINE)
(2) 1.25 MGM. O.D.
(16) 1.25 MGM. B.I.D.
ETHINYL ESTRADIOL
(2) 0.05 MGM. O.D.
(12) 0.05 MGM. B.I.D.
ESTRADIOL
(8) 1.0 MGM. B.I.D.
(2) 2.0 MGM. B.I.D.
TACE
(8) 25 MGM. T.I.W.
(4) 12 MGM. O.D.
(2) 12 MGM. B.I.D.
ESTRADIOL & ETHINYL ESTRADIOL
(1) 0.4 MGM. ESTRADIOL 0.04 MGM. ETHINYL ESTRADIOL O.D.
(6) 0.4 MGM. ESTRADIOL 0.04 MGM. ETHINYL ESTRADIOL B.I.D.
0 1 2 3 4 5
AMENORRHEA INDEX
(FIGURE IN PARENTHESIS = NUMBER OF TRIALS)

FIGURE 5

DISCUSSION

By employing these two systems of classification, *i.e.*, the amenorrheal and menopausal indices, one can evaluate an estrogenic compound as to its efficacy in producing withdrawal bleeding and in controlling the climacteric symptomatology. It is important to note that evaluation of the estrogens in the menopausal series by use of the menopausal index requires a large number of patients so that a statistical analysis can be attained. In the event that the number of patients is few, the data cannot very well be subjected to the menopausal index type of evaluation. Only with large numbers of patients can one estrogenic compound be compared with another and only by use of blind testing procedures are objective methods of evaluation obtainable (4). It is important that the interrogator be unaware of the preparation which is being used so that he is not influenced in judging the clinical response by his own opinion of the effectiveness of the estrogenic preparation received by the patient. Adequate control of observations on the use of any compounds in the menopause can only be obtained by adequate use of placebos.

The amenorrheal index, on the other hand, is a more objective means of classification, although it is dependent, in part, upon the patient's judgment of the extent of flow. However, this possible error is diminished

very markedly if we consider the fact that the extent of bleeding is only one factor in the amenorrheal index. The quotient obtained by dividing the duration of withdrawal bleeding in days by the time required for onset of bleeding in days is a much more vital factor in obtaining the so-called amenorrheal index than is the actual extent of flow. The amenorrheal index, when progesterone is employed, will give more reliable data for evaluating estrogenic preparations that may have a sustained or prolonged effect. Such estrogens, when given alone without progesterone may not necessarily cause withdrawal bleeding, since the effect of the estrogen is long continued and there is no marked decrease or diminishing effect of the estrogen in the organism. However, when progesterone is added to this regimen, regardless of the prolongation of action of the estrogen, the progestational steroid will achieve an effective withdrawal bleeding if the endometrium has been adequately primed by previous administration of estrogen.

Comparison of the relative efficacy of different estrogens by the two procedures gives remarkably consistent results. Ethinyl estradiol, conjugated estrogens (equine), and combinations of ethinyl and free estradiol, yielded the greatest degrees of effectiveness in both indices. The crystalline estrone sulfate compounds of either piperazine or potassium derivatives were inferior to the foregoing compounds by both indices. One note of interest can be made with respect to dinestrol. This preparation in the dose employed showed an amenorrheal index of 2.8, which would imply that this stilbene derivative has an excellent endometrial growth potentiality. TACE, described as a pro-estrogen, is capable of inducing satisfactory endometrial stimulation as indicated by the amenorrheal index noted after progesterone administration. Since TACE is considered as an estrogen with a prolonged effect, only by the use of progesterone can one demonstrate adequate uterine withdrawal bleeding.

The combined evaluation of estrogens by the amenorrheal and the menopausal indices has proved to be an effective means of comparing different estrogenic preparations in the clinic. Both procedures have been developed in an attempt to place estrogenic evaluation in menopausal and in amenorrheal individuals upon an objective foundation, uninfluenced by impressionism based upon subjective empiricism.

SUMMARY

The comparative efficacy of steroidal and nonsteroidal estrogens has been studied in the human by means of the menopausal and amenorrheal indices. The menopausal index represents a numerical conversion factor of the severity of the eleven most common menopausal symptoms.

Eighteen different preparations were used in comparative formulation of the menopausal index, including most of the oral estrogenic preparations available plus vitamin E, phenobarbital and placebo tablets. Based upon changes induced in the menopausal index and the dose employed, the following order of effectiveness was observed with the various preparations: Ethinyl estradiol, conjugated estrogens (equine), and ethinyl estradiol plus free estradiol were the most effective. Vitamin E was no more effective than a placebo in the cases studied. Phenobarbital was more active than a placebo in controlling the menopausal symptoms. Various crystalline estrone sulfate compounds, including a piperazine derivative, were not as effective as equivalent doses of conjugated estrogens (equine). Stilbestrol, dinestrol, hexestrol and TACE, together with estradiol linguents and tablets and equine estrogenic factors, were intermediate in activity between the first three groups of drugs and phenobarbital.

Evaluation of these same compounds by the amenorrheal index confirmed our observations on the order of activity of the same estrogens, assayed by the menopausal index.

The amenorrheal index represents the numerical conversion of the degree of withdrawal bleeding occurring after the patient has received therapy for three weeks with the estrogen to be tested, followed by a single intramuscular injection of 100 mg. of progesterone. The factor is obtained by dividing the duration of bleeding in days by the time required for onset of bleeding in days after the administration of progesterone, and multiplying by the extent of bleeding. Ethinyl estradiol, alone and in combination with free estradiol, and conjugated estrogens (equine) proved to be the most effective estrogens when administered in twice the daily dose used in obtaining the menopausal index.

The use of these two indices has resulted in reproducible hormone assay procedures for the comparative evaluation of estrogens in the human.

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Ethinyl estradiol (Estinyl), estradiol linguets (Progynon buccal tablets), estradiol tablets (Progynon tablets), and potassium estrone sulfate were furnished through the courtesy of Dr. Norman Heminway of the Schering Corporation.

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SOME METABOLIC AND BIOCHEMICAL EFFECTS OF METHYLANDROSTENEDIOL*

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INTEREST in methylandrostenediol (MAD)[1] has recently been increased because of evidence that this agent, which is only weakly androgenic, promotes tissue synthesis but causes no retention of salt or water (1, 2). Clinical trial has further shown that it is useful as a palliative agent in the therapy of advanced carcinoma of the breast (3). The present study deals with some of the metabolic and biochemical changes observed in 50 women with cancer of the breast who received MAD for prolonged periods of time, and in 4 additional patients on whom metabolic balance studies were carried out.

METHODS

The patients were continuously hospitalized for from one to eighteen months and received from 26 to 80 Gm. of MAD by mouth and/or parenterally. Their ages ranged from 28 to 74 years, and averaged 54.8 years. The clinical histories of some of these patients have been presented elsewhere (3). The group with carcinoma of the breast all had soft tissue or bone metastases or both, and their condition ranged from moderately ill to completely bedridden.

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[1] Methylandrostenediol (MAD) designates 17α-methyl-Δ^5-androstene-3β,17β-diol (Mestenediol), obtained through the courtesy of Organon, Inc., Orange, N. J. under the trade name of Stenediol, and from the Schering Corp., Bloomfield, N. J. under the trade name of Methostan.

Metabolic studies were made in 1 patient with cancer of the breast, in 2 with rheumatoid arthritis and in 1 with seborrheic dermatitis. Of the 2 patients with rheumatoid arthritis, one (D.L.) was a woman who had moderately severe disease and was semiambulatory; the other (B.L.) was a man who had transverse myelitis due to a chordoma as a complication of mild rheumatoid arthritis, and was completely immobilized in bed.

The methods for the metabolic balance studies were those described elsewhere (4). The metabolic periods and dosages used are shown in the results. In 1 case (D.L.) a comparative study with testosterone propionate was conducted. In 1 of the patients (B.L.) with rheumatoid arthritis and chordoma the administration of ACTH alone and in combination with MAD was used to measure the ability of MAD, administered by mouth, to overcome the protein catabolic effects of ACTH.

Urinary 17-ketosteroid excretion was studied regularly in Patient B.L., and also in 3 young males with neurologic diseases, for four months at weekly intervals before and during administration for three months of 300 mg. of MAD daily by mouth.

The concentrations of plasma proteins and of serum calcium, phosphorus, alkaline phosphatase and nonprotein nitrogen were determined at regular intervals. The patients were weighed at least once a week, when possible. Repeated skeletal roentgenograms were obtained in all cases.

RESULTS

1. *Protein metabolism*

Table 1 summarizes the effects of MAD given intramuscularly in aque-

TABLE 1. SUMMARY OF NITROGEN BALANCE DATA

Patient	Sex	Age (yrs.)	Diagnosis	Medication (daily)	Metabolic period (days)	N retained (+); N lost (−); (mg./Kg./dy.)	Level of N intake (Gm./dy.)
D. L.	F	46	Rheumatoid arthritis	None;	18	−20	8.5
				MAD 12.5 mg.	6	+40	9.8
				MAD 25 mg.	6	+37	9.8
				Testosterone prop. 25 mg.	3	+100	8.7
A. S.	F	46	Carcinoma of breast	None	18	−8.3	7.0
				MAD 100 mg.	3	+16	6.7
H. Y.	M	52	Dermatitis	None	6	+8.2	13.2
				MAD 100 mg.	9	+8.7	13.2

ous suspension to 3 patients exhibiting varying degrees of malnutrition during relatively short metabolic periods. The 2 patients in negative nitrogen balance prior to medication, demonstrated a significant retention of nitrogen during MAD therapy.

Comparison of the effects of testosterone propionate and methylandrostenediol in D.L. showed that the former caused considerably more nitrogen retention.

In the third subject (H.Y.), a patient with seborrheic dermatitis who was in positive nitrogen balance to begin with, MAD did not have any nitrogen-retaining effect.

Figure 1 summarizes the metabolic balance data obtained in a male patient (B.L.) aged 58 with mild rheumatoid arthritis and a transverse myelitis due to a chordoma. This chart shows the values for nitrogen balance and phosphorus balance, plotted according to the method of Reifenstein *et al.* (5). Data on urinary 17-ketosteroids, circulating eosinophils, sedimentation rate, and creatinine excretion are also shown.

ACTH alone, 100 mg. daily, caused an increase in the excretion of nitrogen. The addition of 300 mg. per day of oral MAD was accompanied by the following changes:

1. There was significant retention of nitrogen, which exceeded the retention during the control period, even though ACTH was continued during MAD administration.

2. After MAD had been given for eighteen days, immediately following its discontinuance there was a "rebound" phenomenon characterized by a return to a negative nitrogen balance.

3. There was a significant drop of the urinary creatinine excretion during the first nine days of MAD administration, with creatinine levels returning towards normal when ACTH was omitted and while MAD was still being given.

4. The urinary excretion of 17-ketosteroids, which increased when ACTH was being given, declined during the administration of MAD. There was a short febrile episode during periods 11 and 12, due to a urinary infection. This may have caused the rise in 17-ketosteroid excretion during these periods.

Of 11 patients with cancer of the breast studied, 2 showed a significant increase in the level of plasma proteins (over 2 Gm./100 ml.) which coincided with other objective signs of improvement, such as weight gain without edema, and demonstrable recalcification of bone lesions. There was no evidence that the increased plasma protein levels were due to plasma concentration by dehydration.

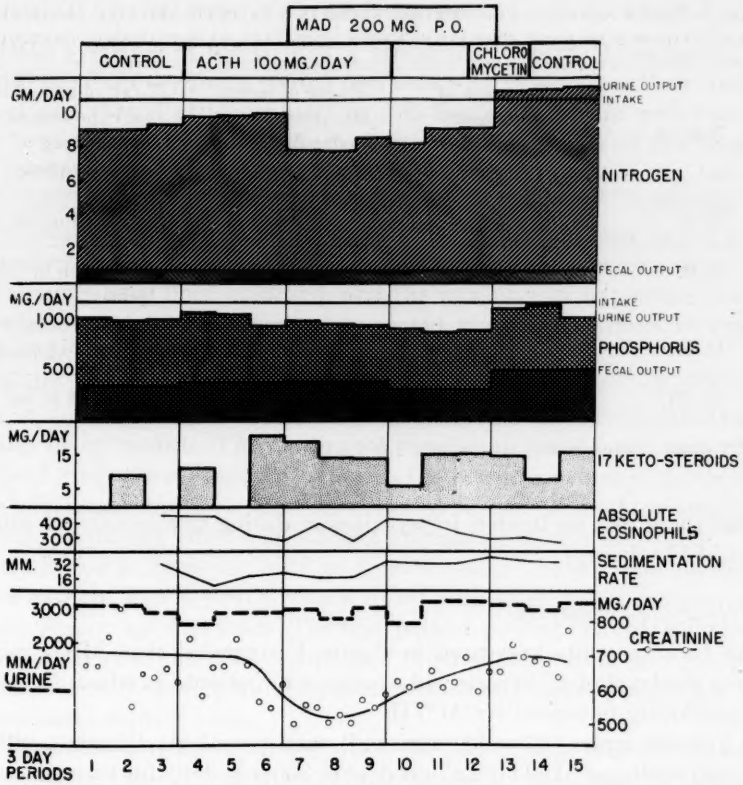

FIG. 1. Data obtained in a white man of 58 years of age, long bedridden with mild rheumatoid arthritis, and paraplegic because of a chordoma (*see* discussion in text).

2. *Calcium metabolism and alkaline phosphatase* (*Table 2*)

There was no significant increase of the alkaline phosphatase in the 4 patients with cancer of the breast who showed objective responses, such as x-ray evidence of bone calcification, while receiving MAD. In fact, 3 of these 4 patients showed a fall of the alkaline phosphatase from a normal or slightly elevated level, to a low level. The concentrations of serum calcium and phosphorus showed no significant relation to either hormone therapy or clinical response.

There was 1 patient in whom hypercalcemia developed while receiving MAD. Another patient was admitted in coma due to spontaneous hypercalcemia, and recovered following treatment with sodium citrate. In this

TABLE 2. SERUM ALKALINE PHOSPHATASE LEVEL IN 4 PATIENTS SHOWING CLEAR-CUT X-RAY EVIDENCE OF BONE FORMATION WHILE RECEIVING METHYLANDROSTENEDIOL

Patient	Duration of treatment with MAD (days)	Range of serum alk. phosphatase (Bodansky units)* during treatment	X-ray observation on bone lesions showing calcification
H. S.	360	0.3–3	(*See* Fig. 6 in ref. 3)
S. B.	60	3.2–4.3	Calcification of costal lesions
C. S.	180	1.3–2.2	(*See* Fig. 5 in ref. 3)
M. M.	450	1.8–5.4	(*See* Case 8 in ref. 3)

* "Normal" range in this laboratory, 1.0–4.5 units.

patient there was no further hypercalcemia during five months of subsequent MAD therapy.

3. *Urinary 17-ketosteroids*

The balance study presented in Figure 1 suggested that MAD might depress the level of 17-ketosteroid excretion in patients in whom the level was previously increased by ACTH.

In 3 young males (25 to 38 years old) with neurologic disease (multiple sclerosis) receiving MAD in an oral dose of 300 mg. daily for three months, no significant changes in the 17-ketosteroid excretion were seen during therapy. Before treatment the 17-ketosteroids ranged from 6.34 mg. to 10.40 mg. and during treatment from 4.91 to 16.40 mg. per twenty-four hours.

4. *Other clinical effects of MAD*

In 1 case moderate hirsutism was seen in a woman of 48 years of age. This appeared during the first month of therapy and slowly progressed until the patient was definitely, though not markedly, hirsute. In 2 other women, aged 73 and 74, some downy hair appeared on the face during therapy but this remained slight, even though therapy was continued for more than a year. In 1 of these women a mild seborrhea of the face also developed. No enlargement of the clitoris, change of voice, or acne was encountered. In none of these patients was there any clinical evidence of salt or water retention.

DISCUSSION

Data available at the time of a preliminary report (1) suggested that MAD is a "non-virilizing steroid hormone with moderate protein anabolic effect and that it causes subjective well-being in patients with cancer of the breast." The palliative effect of this agent in cancer of the breast in 50 patients observed during an additional twenty-five months, has been substantiated (3). There has been, however, evidence indicating that occasionally the compound produces mild virilization, but never to the extent encountered with testosterone. Except for hypercalcemia, no toxic phenomena have been observed, and the incidence of hypercalcemia was no higher than would have been expected without treatment in a group of patients such as the one receiving this medication.

In the patients investigated while on a nitrogen balance regimen, there was an increased retention of nitrogen when MAD was given either parenterally or by mouth. This finding of significant protein anabolic effects without virilization is at variance with the recent conclusion of others (8) that "it seems probable that in doses sufficient to produce significant growth promoting effects, significant androgenic effects also occur." The contradiction is, however, only an apparent one. The subjects studied by these authors were a young female dwarf with panhypopituitarism and 2 young women aged 28 years. The first patient presented a marked aberration from normal; and the second and third patients were in a group notoriously more sensitive to virilization because of relative youth than were the subjects studied by us.

In metastasizing cancer of the breast the beneficial effects of MAD seem to be related more closely to its protein anabolic effects than to its androgenic activity. In this connection it is of interest to note that in 2 patients with breast cancer and bone metastases who showed maximal palliative effects from MAD, there was a significant rise in the level of plasma protein, a phenomenon which occurs but rarely with testosterone propionate. Definite radiologic signs of bone formation were the only measurable evidence of an effect of MAD on calcium metabolism. This osteoblastic activity was not reflected by a change in the level of serum alkaline phosphatase, a finding which is customarily regarded as a reliable laboratory index of osteogenesis. Serum calcium and phosphorus values showed insignificant fluctuations (except in the 1 case of hypercalcemia).

The urinary 17-ketosteroids did not increase in 3 young men with neurologic disease during the oral administration of 300 mg. of MAD per day. This was in accord with the findings of Segaloff *et al.* (6) demonstrating the absence of an increased 17-ketosteroid excretion in women with cancer of the breast receiving MAD, and with the observation of Querido *et al.*

(7) that the end-products of MAD metabolism were not of a 17-ketosteroid nature. This effect is different from that of testosterone propionate and of dehydroepiandrosterone, which caused elevation of the urinary ketosteroids ranging from 2 to 13 times the normal level (9).

In 1 patient (B.L.) in whom ACTH caused the usual increase of urinary 17-ketosteroids, MAD seems to have counteracted the adrenal stimulation, as indicated by the lowered 17-ketosteroid excretion. This effect may be similar to that of MAD in mice. In these animals the weights of the adrenal glands have been decreased by administration of MAD (10).

SUMMARY

Metabolic balance studies and investigation of some of the biochemical effects of methylandrostenediol (MAD) in patients with rheumatoid arthritis, cancer of the breast and other diseases have shown:

1. In patients with negative nitrogen balance, MAD caused retention of nitrogen.
2. MAD counteracted the protein catabolic effect of ACTH and reduced the urinary steroid excretion that had been increased by ACTH.
3. In young men with neurologic disease, 300 mg. of MAD daily by mouth did not increase the excretion of 17-ketosteroids.
4. In patients having definite radiologic evidence of osteogenesis in the course of MAD treatment for bone metastases of breast cancer, there was no elevation of the serum alkaline phosphatase level.
5. Significant and persistent increases of the plasma protein level were seen in 2 patients obtaining satisfactory palliation of metastasizing cancer of the breast.
6. The impression has been gained that the beneficial effects of MAD in cancer of the breast are related more closely to its protein anabolic effects than to its androgenicity.

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THE INFLUENCE OF ADRENAL AND GONADAL STEROIDS ON THE UPTAKE OF IODINE BY THE THYROID GLAND

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IN RECENT years there has been a renewal of interest in the relationship between the hormones of the thyroid and the adrenal cortex. It has been generally established that cortisone and ACTH have a depressing action on thyroid function in man, as measured by the thyroid uptake of radioiodine (1–4) and by the plasma organic iodine level (2, 5). The thyroid inhibitory action of ACTH and cortisone has also been studied in the rat (6–9). There are a few reports not in accord with the concept of cortical hormone inhibition of the thyroid (10, 11).

There has been relatively little reported on the effect of other steroid hormones on thyroid function, especially in man, and the reported results are conflicting. Desoxycorticosterone has been claimed to have no effect on the thyroid of the intact rat (10) but has also been shown by others to increase the thyroid iodine concentration in both adrenalectomized (12) and normal rats (13), as well as in guinea pigs (14). Evidence has been presented in favor of an ovarian-thyroid antagonism (15) but both ovariectomy and estrogen administration have failed to influence the ability of the thyroid to take up radioiodine (16). However, Money *et al.* (6) have reported increased collections of iodine by the rat thyroid following treatment with estrone, progesterone and testosterone and decreased collections after estradiol and pregnenolone.

This communication presents additional data confirming the inhibitory action of cortisone on one aspect of thyroid function in the human, and observations on the influence of desoxycorticosterone, estradiol, progesterone and testosterone on the ability of the thyroid to take up iodine.

SUBJECTS AND METHODS

The subjects are listed in Table 1. The majority were patients on the psychopathic ward of the Winnipeg General Hospital and had no organic diseases nor had they undergone shock therapy, surgery or endocrine therapy within at least a year prior to being studied. As far as could be

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ascertained, none of the patients had been taking iodine in amounts other than encountered in ordinary diets. A few of the patients were from other services but had no thyroid, adrenal or renal disorders, had been given normal diets and had received no iodine-containing medication.

TABLE 1. CLINICAL DETAILS

Subject	Sex	Age (yrs.)	Treatment	Diagnosis
T.K.	M	18	DCA	Tuberculosis of ankle
W.O'C.	M	38	DCA	Psychosis with mental deficiency
D.C.	M	22	DCA	Schizophrenia affective psychosis
G.S.	M	50	DCA	Mental deficiency with psychotic episodes
R.A.	M	27	DCA	Schizophrenia, paranoid
H.B.	F	41	DCA	Neurotic depressive reaction
W.P.	M	17	DCA	Primary behavior disorder
E.L.	F	22	DCA	Psychosis, mental deficiency
A.S.	F	21	DCA	Schizophrenia affective psychosis
H.M.	F	21	DCA	Schizophrenia, paranoid
T.T.	M	31	DCA	Chronic alcoholism*
R.R.	M	35	DCA	Parkinsonism, postencephalitic
R.F.	F	51	Cortisone	Rheumatoid arthritis
J.O'L.	M	32	Cortisone	Schizophrenia, paranoid
L.R.	M	39	Cortisone	Manic depressive; depressed
M.C.	M	31	Cortisone	Manic depressive; manic
P.E.	M	39	Cortisone	Simple schizophrenia
D.E.	F	23	Cortisone	Psychoneurosis, anxiety state
J.S.	M	17	Estradiol	Schizophrenia, catatonic
A.L.	M	43	Estradiol	Schizophrenia, paranoid
W.M.	M	27	Estradiol	Chronic alcoholism*
C.M.	M	23	Progesterone	Incipient schizophrenia
A.B.	M	49	Progesterone	Immature personality, emotional instability
J.Y.	M	20	Progesterone	Psychoneurosis, anxiety state
S.M.	M	26	Testosterone	Simple schizophrenia
J.M.	M	19	Testosterone	Schizophrenia, catatonic
C.S.	M	31	Testosterone	Schizophrenia, paranoid
V.R.	F	32	Testosterone	Simple schizophrenia

* Off alcohol for several weeks.

The same procedures were carried out twice on all patients—once before, and once after treatment with the various steroids. Each patient thus acted as his own control. At 7 A.M. after a fourteen-hour fast the subject was given 50–150 microcuries of radioiodine (I^{131}) orally. Approximately two hours later the bladder was emptied, the exact time noted and almost simultaneously a blood sample was taken and counts made over

the thyroid area to measure the thyroid uptake of iodine. Again, approximately two hours later, these procedures were repeated and the exact time noted; the urine passed during this exactly timed period was collected and saved. The patients carried out their usual ward activities but were allowed no food during the four-hour period. They were kept well hydrated, 200 ml. of water being given to drink approximately every thirty minutes.

Twenty-six hours after the ingestion of the radioiodine the thyroid uptake was again determined and a third blood sample taken. The thyroid uptake was also determined after a further 24-hour interval—fifty hours after the dose. Following the fifty-hour count, treatment was begun with the various steroids. After three full days of steroid treatment the foregoing procedures were repeated, the administration of the hormones being continued during this time.

At the time of the second dose of radioiodine there was an appreciable amount of radioactivity still in the thyroid. This amount was determined just prior to the administration of the second dose of the isotope and deducted from the ensuing thyroid counts. After appropriate correction for physical decay, this factor was also deducted from the 26-hour and 50-hour values. The difference between the effective half-life and the physical half-life following the third day after a tracer dose is small (17), and to reduce further any error from residual iodine from the first dose, the second dose of the isotope was always at least twice the first one.

The amount of radioactivity present in the thyroid was measured by means of a shielded, collimated scintillometer,[1] readings being taken six inches above the thyroid isthmus and compared with similar readings taken six inches above a 25-ml. glass bottle containing an aliquot of the dose of I^{131} given the patient; in this way the thyroid I^{131} was expressed as a percentage of the given dose. Thyroid counts were corrected by subtracting a background count taken six inches over the mid thigh, and the standard counts were corrected by subtracting the background counts of the experimental room.

The blood samples were collected in heparin and the plasma immediately separated. The radioactive iodine concentrations in plasma and urine were measured by procedures previously described (18). Protein-bound plasma iodine and urinary stable iodine (I^{127}) estimations were carried out by a modification of Barker's method (19).

From the amounts of radioiodine in the plasma determined at two known times, and from the amount of I^{131} taken up by the thyroid gland and excreted in the urine during this known time interval, it is possible to calculate the rates of clearance of I^{131} from plasma by the thyroid and kidneys, respectively. The mean I^{131} concentration in the plasma during the two-hour clearance period was calculated, using the formula of Keating *et al.* (20) $P_1 - P_2/\log_e P_1 - \log_e P_2$. Endogenous creatinine clearance was determined as a measure of glomerular filtration rate.

Renal clearance was calculated from the usual formula, UV/P, and thyroid clearance from the formula, thyroid uptake per minute/P, where P is the mean concentration described above. Values for the renal clearance of creatinine and iodide were standardized

[1] Scintillometer, Model #939. Halross Instrument Corporation, Winnipeg, Canada.

to a body surface of 1.73 m^2, this estimation being based on the patient's height and weight during the first clearance period.

The inorganic stable iodine (I^{127}) concentration of plasma during the clearance period was calculated, using the method of Stanley (21) which assumes complete mixing of the two isotopes, so that the ratio I^{131} concentration/I^{127} concentration in urine is the same as that in plasma. Thus, knowing the urinary I^{131} and I^{127} concentrations and the plasma I^{131} concentration, plasma I^{127} concentration is readily calculated and similarly a calculation of the thyroid I^{127} uptake may be made.

Total plasma protein determinations were made on the subjects by the method of Phillips *et al.* (22).

The influence of the steroids desoxycorticosterone (DCA), cortisone, estradiol, testosterone and progesterone were investigated, the administration being as follows:

Desoxycorticosterone (Percorten-Ciba)
10 mg. daily i.m. in two 5-mg. doses twelve hours apart, for six days
Cortisone acetate (Cortone-Merck for oral use)
Day 1 250 mg. in three divided doses eight hours apart
Day 2 200 mg. in four divided doses six hours apart
Day 3 150 mg. in three divided doses eight hours apart
Day 4 250 mg. in three divided doses eight hours apart
Days 5, 6, 7 150 mg. in three divided doses eight hours apart
Testosterone (Perandren-Ciba)
25 mg. daily i.m. in two doses twelve hours apart, for six days
Progesterone (Lutocylin-Ciba)
25 mg. daily i.m. in two doses twelve hours apart, for six days

Statistical analysis of the results obtained with DCA and cortisone were by the method "significance of the mean of a small sample" described by Fisher (23). Because there were only a few persons so investigated, statistical analysis of data from those treated with estradiol, testosterone and progesterone was not made.

Results

Effect of DCA on thyroid function

In Figure 1 the per cent of the dose of radioiodine present in the thyroid at various times is compared in 12 subjects before and after treatment with DCA. In all patients the percentage uptake was lower after the treatment at each time, with the exception of 2 patients (T.K. and D.C.) whose 26-hour and 50-hour values respectively, were slightly increased after DCA.

In Table 2 are shown the values for the thyroid accumulation rate of both radioactive and stable iodine and the thyroid clearance rate of radioiodine in the same 12 patients before and after administration of DCA. Also shown in this table are the calculated plasma iodide^{127} level, the

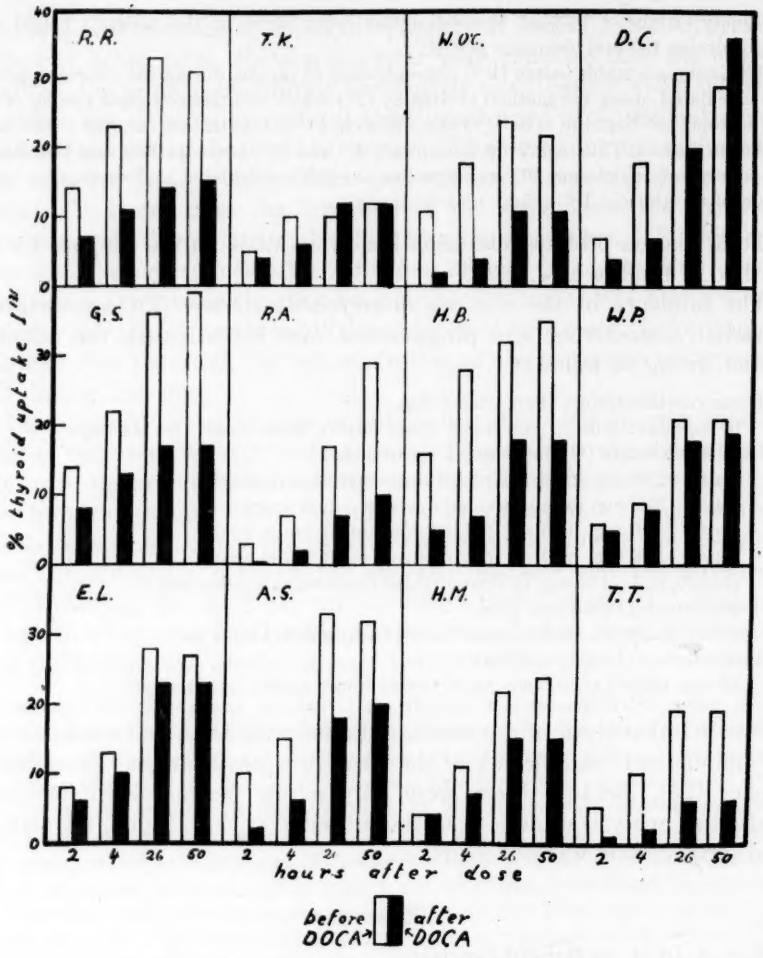

FIG. 1. The thyroid uptake of I^{131} before and after treatment with desoxycorticosterone.

renal clearance rate of I^{131}, the plasma protein-bound iodine concentration and the total plasma protein concentration.

As may be seen there was a marked depression of thyroid function, as measured by iodine uptake and I^{131} clearance after three days of treatment with 10 mg. of DCA per day. Although the plasma level of I^{127} tended to rise, the uptake of stable iodine by the thyroid was depressed. Both the protein bound iodine and total plasma proteins were lower after DCA, the

TABLE 2. THYROID FUNCTION BEFORE AND AFTER TREATMENT WITH DCA

Subject	Thyroid uptake of I^{131} (%/hr.)		Thyroid uptake of I^{127} (μgm./hr.)		Thyroid clearance of I^{131} (ml./min.)		Renal clearance of I^{131} (ml./min.)		Plasma I^{127} (μgm./100 cc.)		PBI (μgm./100 cc.)		Plasma protein (Gm./100 cc.)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
T.K.	1.7	0.9	1.8	0.5	10.6	2.0	36.5	27.4	0.28	0.30	4.9	—	—	—
W.O'C.	3.6	0.9	1.1	0.6	21.8	6.0	40.6	57.0	0.09	0.17	3.5	—	—	—
D.C.	5.5	2.3	3.3	2.5	30.5	11.5	39.8	37.3	0.18	0.36	3.2	—	—	—
G.S.	4.4	2.2	1.5	0.6	23.4	10.5	36.9	37.0	0.10	0.10	6.7	—	—	—
R.A.	1.7	0.7	2.7	1.8	7.1	3.4	22.9	26.2	0.63	0.87	4.2	—	—	—
H.B.	5.6	1.2	2.7	1.2	24.2	6.5	21.7	13.0	0.18	0.34	4.8	3.7	6.4	5.7
W.P.	1.8	1.6	0.9	0.6	9.5	8.9	22.6	33.0	0.15	0.12	5.3	4.0	7.2	6.3
E.L.	2.0	2.2	—	—	14.9	16.5	28.9	7.5	—	—	8.8	5.7	6.3	5.8
A.S.	2.5	2.1	2.4	2.3	16.1	12.3	21.6	20.7	0.25	0.31	5.5	6.0	6.9	6.7
H.M.	4.1	1.3	4.1	1.3	17.9	6.8	19.3	25.5	0.26	0.21	8.3	7.5	6.8	6.2
T.T.	2.3	0.4	2.3	0.4	11.5	2.7	24.6	36.6	0.25	0.72	7.0	7.0	6.6	6.5
R.R.	3.5	2.1	—	—	20.6	2.6	—	—	—	—	—	—	—	—
Mean± S.E.	3.2± 0.4	1.5± 0.2	2.3± 0.3	1.2± 0.3	17.4± 2.0	7.5± 1.3	28.8± 2.6	28.7± 4.2	0.25± .03	0.31± .07	6.6± .7	5.5± .7	6.7± 0.1	6.0± 0.2
Mean difference± S.E.	±1.7 ±0.4		−1.1 ± .25		−9.9 ±2.0		− .1 ±3.1		−0.6 ±0.4		−1.1 ±0.7		−0.7 ±0.1	
P value	<.01		<.01		<.01		>.05		>.05		>.05		<.05	

decrease in plasma proteins being significant. Some patients showed a slight increase in body weight under the influence of DCA. The renal clearance of I^{131} was not significantly altered by DCA and hence it would appear that the lowered uptake of iodine by the thyroid was not a result of an increased excretion by the kidney.

Effect of cortisone on thyroid function

The action of this steroid was observed in 6 subjects. The thyroid uptakes at two, four, twenty-six and fifty hours after administration of I^{131}, before and after cortisone, are shown in Figure 2. The results obtained were similar to those noted following DCA—a decrease in the percentage of the dose taken up by the thyroid gland at these time intervals. In 2 subjects the two-hour uptakes were only slightly lower after cortisone but the 4-hour, 26-hour and 50-hour uptakes were much depressed.

In Table 3 are shown the various values for these 6 patients before and after three days of treatment with cortisone. Cortisone depressed the thyroid accumulation rates of both stable and radioactive iodine, and depressed the thyroid clearance rate of I^{131}, the depression of the clearance rate and accumulation rate of I^{131} being highly significant. As with DCA, the renal clearance rate of I^{131} was unaffected by cortisone. Similarly, no alterations occurred in the plasma level of I^{127}, the plasma protein concentration, or the protein-bound iodine levels. The body weight changes were negligible.

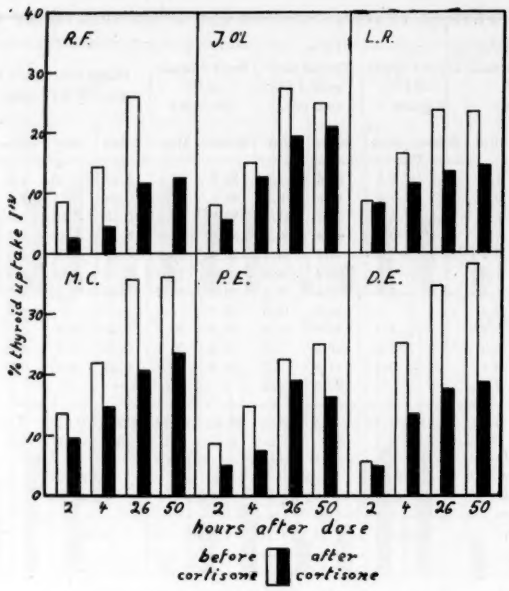

FIG. 2. The thyroid uptake of I^{131} before and after treatment with cortisone.

TABLE 3. THYROID FUNCTION BEFORE AND AFTER TREATMENT WITH CORTISONE

Subject	Thyroid uptake of I^{131} (%/hr.)		Thyroid uptake of I^{127} (μgm./hr.)		Thyroid clearance of I^{131} (ml./min.)		Renal clearance of I^{131} (ml./min.)		Plasma I^{127} (μgm./100 cc.)		PBI (μgm./100 cc.)		Plasma protein (Gm./100 cc.)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
R.F.	2.7	1.0	1.6	0.3	14.4	8.3	38.8	32.5	0.18	.06	3.0	3.5	6.4	6.3
J.O'L.	3.6	2.7	3.8	2.0	24.8	22.6	26.2	—	0.25	0.15	3.3	3.5	6.2	6.7
L.R.	3.8	1.5	1.8	1.5	18.3	11.4	29.4	38.5	0.17	0.22	3.3	3.3	5.8	6.0
M.C.	4.2	2.3	2.7	1.7	22.1	18.3	18.9	11.7	0.20	0.16	3.6	3.8	5.7	5.8
P.E.	2.9	1.0	5.0	2.0	17.2	5.2	27.7	15.7	0.48	0.66	4.2	4.2	6.9	6.7
D.E.	6.2	4.3	1.6	1.6	31.9	26.4	31.1	44.8	0.09	0.10	4.5	4.5	6.9	7.1
Mean ± S.E.	3.9 ±0.6	2.1 ±0.6	2.8 ±0.6	1.5 0.3	21.5 ±2.8	15.4 3.4	29.2 ±3.1	28.6 ±4.1	0.23 ± .06	0.23 ± .09	3.5 —	3.6 —	6.3 —	6.4 —
Mean difference ± S.E.	−1.8 ± .2		−1.2 ±0.4		−6.1 ±1.4		−0.6 ±3.5		0		0		0	
P value	<.01		<.01		<.01		>.05							

Effect of estradiol, progesterone and testosterone

This group consisted of 10 subjects; 3 were treated with estradiol, 3 with progesterone and 4 with testosterone. It will be seen in Table 4 that estradiol produced changes difficult to interpret, for although the accumulation rate of I^{131} was depressed in all 3 subjects, the changes in uptake of stable iodine were inconsistent, being increased in one, depressed in another and unchanged in the third. Similarly the thyroid clearance of I^{131} was depressed in the first subject, unchanged in the second and somewhat elevated in the third. In Subject J.S., who showed an increased I^{127} uptake and elevated plasma I^{127} following estradiol, ingestion of iodine between the two clearance periods was suspected.

TABLE 4. THYROID FUNCTION AFTER TREATMENT WITH ESTRADIOL (E), PROGESTERONE (P) AND TESTOSTERONE (T)

Subject	Treatment	Thyroid uptake of I^{131} (%/hr.)		Thyroid uptake of I^{127} (μgm./hr.)		Thyroid clearance of I^{131} (ml./min.)		Renal clearance of I^{131} (ml./min.)		Plasma I^{127} (μgm./100 cc.)		PBI (μgm./100 cc.)		Plasma protein (Gm./100 cc.)	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
J.S.	E	4.0	2.1	1.6	6.6	29.8	17.7	—	—	0.09	0.63	4.2	3.9	6.3	6.5
A.L.	E	2.3	1.7	1.2	0.7	9.4	9.7	—	—	0.21	0.13	4.1	3.0	6.2	6.0
W.M.	E	2.1	1.6	2.5	2.3	10.6	13.0	—	—	0.38	0.29	3.0	4.2	5.8	5.8
C.M.	P	2.2	0.6	1.8	0.7	7.1	3.2	21.6	32.8	0.42	0.34	4.0	3.5	6.0	6.0
A.B.	P	1.5	1.0	1.8	1.2	8.0	7.0	22.9	32.0	0.39	0.33	3.8	3.3	6.7	6.7
J.Y.	P	2.0	1.2	1.5	1.1	10.5	4.4	29.1	16.1	0.27	0.41	5.0	4.2	7.3	6.9
S.M.	T	2.9	2.9	—	—	19.0	16.6	—	—	—	—	4.3	4.4	6.5	6.4
J.M.	T	2.6	3.1	—	—	20.6	19.6	—	—	—	—	3.7	4.7	6.7	6.4
C.S.	T	4.5	2.8	3.2	2.6	37.6	15.6	—	—	0.16	0.28	3.7	3.6	6.1	6.0
V.R.	T	1.6	1.3	0.4	1.1	5.6	4.2	—	—	0.10	0.45	6.6	7.4	6.5	6.5

The accumulation rates of both iodine isotopes, and the thyroid clearance of radioiodine tended to fall after progesterone; the changes in plasma concentration were inconsistent.

Due to incomplete urine collections some values were unobtainable in 2 of the subjects treated with testosterone. Thyroid clearance of I^{131} showed a tendency to decrease in the 4 patients but in only 1, C.S., was the uptake of I^{131} during the clearance period also depressed. No consistent alterations in the protein-bound iodine or plasma proteins or the renal clearance of iodine were observed after estradiol, progesterone or testosterone.

DISCUSSION

Although the majority of the subjects of this study were psychotic individuals, mainly schizophrenics, there is no reason to suppose their thyroid glands reacted to the steroids any differently than those of psychically normal individuals. It has been established (24, 25, 26), using vari-

ous indices of thyroid activity, that the thyroid gland of schizophrenics and other psychotic subjects differs little, if at all, from the normal. Reiss *et al.* (27) postulate a possible thyroid hyperfunction in schizophrenia but our present data and those of others (24, 25) do not support this contention. In addition, the nonpsychotic individuals in our series reacted to the various steroid treatments in a manner similar to that of psychotic subjects. Furthermore, the values for thyroid uptake and clearance of radioiodine obtained in the present series before treatment with steroids did not differ significantly from values obtained on normal euthyroid individuals which have been reported previously (18).

The rate of uptake of both stable iodine and radioiodine and also the rate of clearance of I^{131} from the plasma by the thyroid were markedly depressed by desoxycorticosterone as well as by cortisone. Thus these two adrenal cortical hormones have in common a depressant action on the thyroid, though it appears that on a weight basis, DCA is more potent. The decreased accumulation of radioiodine induced by DCA, as well as indicating a depression of this aspect of thyroid function, could also be a result of a dilution of the I^{131} by retained stable I^{127}, as manifested by the slightly increased plasma levels of I^{127}. However, the simultaneous depression of the rate of thyroid uptake of stable iodine together with the depressed thyroid clearance rate of I^{131}, would indicate a real depression by DCA of the ability of the thyroid gland to concentrate iodine from the plasma. Moreover, similar findings were observed with cortisone and progesterone when there was no suggestion of elevation of the plasma iodide I^{127} level.

No alterations in the plasma level of PBI were noted following cortisone therapy, contrary to other reported findings (1, 2) but in the present experiments the measurements were made after only three days' treatment. The possible slight fall in PBI following DCA may well have been the result of an increase in plasma water which occurs after administration of this substance (28, 29, 30). The slight but significant drop in plasma protein concentration and the slight gain in weight in all those treated with DCA suggests that this dilution occurred. Following cortisone, there was no change in PBI levels, and also no alteration in the concentration of plasma proteins.

The mechanism of the antithyroid action of cortisone is controversial but it appears established that it is not mediated by means of an inhibition of pituitary thyrotropin (8, 9, 31) but possibly by means of an inhibition of thyrotropin at the target-organ level (32). It has been shown in the rat (33) that the mechanism of the inhibiting action of DCA on thyroid uptake of iodine is similar to that reported for cortisone (8) and is not mediated by means of an inhibition of pituitary TSH secretion.

A decreased uptake of iodine by the thyroid could possibly be merely a reflection of an increased renal excretion of iodine but, if so, this should not have affected the thyroid clearance rate of iodine which was also depressed. An increased renal clearance of iodine, paralleling the decreased thyroid uptake rate, has been reported (3) after several days' treatment with cortisone. In the present experiments no such alteration in renal clearance of iodine was noted after three days' treatment with either DCA, cortisone or progesterone.

The numbers of subjects treated with estradiol, progesterone and testosterone were too small to be analyzed statistically. The findings with progesterone suggest that a depression of the thyroid took place comparable to that noted following DCA or cortisone. In view of the close similarity in chemical structure of DCA and progesterone, similar action of these two hormones is perhaps not unexpected. No certain conclusions can be drawn from the observations on the subjects treated with estradiol. Although the thyroid accumulation rate was lower in all 3 patients, this was not accompanied by any definite change in the thyroid clearance rate. Also, in 1 subject the plasma level of stable iodine was so high at the time of the second clearance period that ingestion of some form of iodine was suspected.

Testosterone did not seem to alter thyroid function in 3 of the 4 subjects studied. The fourth subject, C.S., showed some evidence of depressed function, as reflected in both lowered thyroid accumulation and clearance rates.

SUMMARY

Thyroid function has been investigated in 28 subjects before and after receiving either 10 mg. of desoxycorticosterone (DCA), 150–250 mg. of cortisone, 12 mg. of estradiol, 25 mg. of progesterone or 25 mg. of testosterone daily for three days.

The thyroid gland was markedly depressed after administration of either DCA or cortisone, as indicated by lowered uptakes of both stable and radioactive iodine and by a lowered thyroid clearance of I^{131}. As the renal clearance of iodine was unaffected, this lowered uptake of iodine by the thyroid gland seemed to be a true depression of function and not merely a reflection of an increased renal disposal of iodine.

There appeared to be a similar depression with progesterone. Testosterone in the dosage used did not seem to influence the thyroid gland, and the administration of estradiol was followed by inconsistent changes.

Acknowledgments

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USE OF CORTISONE FOR CONGENITAL ADRENAL HYPERPLASIA IN THE MALE: REPORT OF CASE

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CONSIDERABLE interest in congenital adrenal hyperplasia has arisen following the discovery that cortisone inhibits the excessive urinary excretion of 17-ketosteroids that is characteristic of this disorder (1). The published reports, however, have been concerned for the most part with congenital adrenal hyperplasia in the female. It is the purpose of this paper to present observations made during treatment of a 9-year-old boy who exhibited the characteristics of this condition.

REPORT OF CASE

This 9-year-old boy has been observed at the Mayo Clinic since his birth. Delivery was uneventful and no genital abnormalities were noted by the attending pediatricians. Development was not unusual, except that at the age of 1 year he had not yet begun to crawl. At the age of 14 months his penis was noted to be larger than normal and pubic hair was present. The roentgenologic bony age was found to be 2¼ years. Urinary excretion of 17-ketosteroids was 4.1 and 4.5 mg. per twenty-four hours. Because of these findings, the diagnosis of macrogenitosomia praecox resulting from congenital adrenal hyperplasia was made.

At the age of 18 months the penile enlargement was much more apparent. Acne had appeared. The roentgenologic bony age was estimated to be from 2½ to 3 years. Surgical exploration revealed the right adrenal to be as large as, or larger than, that of an adult. Biopsy revealed no definite histologic abnormality.

At the age of 3½ years the roentgenologic bony age was 9 years and the urinary excretion of 17-ketosteroids was 10.2 mg. per twenty-four hours. His height was 46¾ inches (119 cm.), compared to an average height at this age of 39 inches (99 cm.); he weighed 48 pounds (21.8 Kg.), compared to an average of 34 pounds (15.4 Kg.). Roentgenologic study of the head revealed bilateral calcification of the choroid plexuses, but the cause for this was not determined. The I.Q. was 112 as determined by the Stanford-Binet technic. The pubic hair now was well developed, his penis was large and his testes were relatively small.

At the age of 8 years, his roentgenologic bony age was 15 years and his height was 63½ inches (161 cm.), compared to an average of 49 inches (124 cm.) at this age. He weighed 106 pounds (48 Kg.), compared to an average of 53 pounds (24 Kg.). He was somewhat asthenic, as though muscular development had not kept pace with skeletal growth. Scanty growth of beard was noted and the voice was husky. The parents were

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aware of no nocturnal emissions or erections. A light growth of hair was present over the lower part of his abdomen and the pattern and texture of the hair on his legs were adult in nature. No symptoms suggestive of adrenal insufficiency had been present at any time, but his strength was not equal to that of his companions and often he was exhausted at the end of his daily sessions at school.

Therapy with cortisone was begun in March 1951, when the patient was 8 years of age, and was continued for thirteen months. The cortisone was administered orally in divided doses during the period of study, the total daily dose varying between 25 and 75 mg. During this period of treatment, his height increased $1\frac{3}{4}$ inches (4.5 cm.) but his weight did not change. The blood pressure was 124 mm. of mercury systolic and 80 diastolic at the beginning of treatment and did not vary significantly from this value. The bony age was estimated to be 16 years at the end of one year of treatment and it was the opinion of the radiologist that much further growth was unlikely because of beginning epiphyseal fusion at the shoulder and knee.

The patient always had been shy but had adjusted reasonably well to his handicap, although he obviously was aware of his difference from other children. During the period of treatment, it was the opinion of the parents that he had considerably greater strength and endurance. Some decrease in the amount of acne occurred, but otherwise no striking changes were noted clinically. When treatment was interrupted for one week, weakness and lessened activity were noted.

LABORATORY METHODS

Assays for gonadotropins were carried out according to the method of Frank, Salmon and Friedman (2). Estrogens were determined by the method of Albert (3), which is a modification of the methods of Smith and Smith (4) and Lauson and associates (5) in which 1 rat unit is equal approximately to 1 microgram of estrone. Corticosteroids were measured by a modification of the method of Corcoran and Page (6). Determination of 17-ketosteroids was made by the method of Callow, Callow and Emmens (7) and the values were corrected by use of the equation of Fraser and associates (8).

OBSERVATIONS

The results of the hormonal assays during the entire study are recorded in Figure 1. Before treatment, the excretion of 17-ketosteroids varied between 41.1 and 54.0 mg. per twenty-four hours. The range of excretion of 17-ketosteroids when the patient was taking 37.5 mg. of cortisone daily was from 12.1 to 33.2 mg. per twenty-four hours; the range when taking 50 mg. of cortisone daily was from 17.0 to 20.5 mg. Administration of 75 mg. of cortisone daily was capable of maintaining the excretion of 17-ketosteroids between 3.9 and 10.0 mg., although a lag period of ten days was noted before values fell below 10.0 mg.

Excretion of corticosteroids varied between 0.37 and 0.6 mg. prior to treatment. These values are considered to be in the lower range of normal for adults, but are somewhat elevated for a child of this chronologic age.

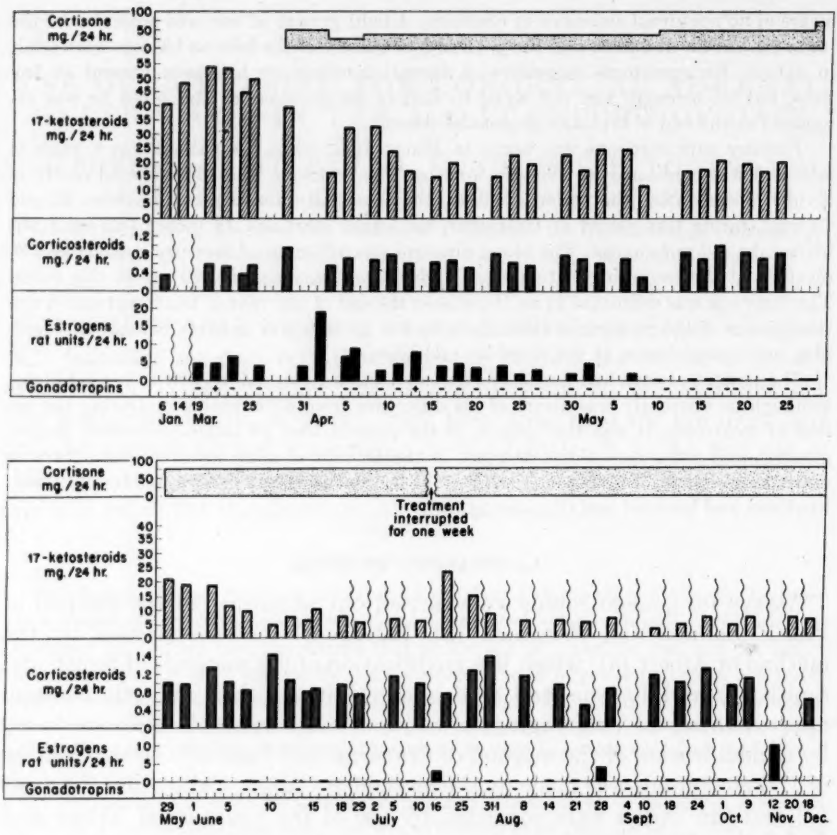

FIG. 1. Hormonal effects of cortisone in macrogenitosomia praecox.

While cortisone was being given, the excretion of corticosteroids was increased appreciably, reaching a maximum of 1.64 mg.

Excretion of estrogens varied between 4 and 7 rat units daily before treatment. These values are considerably greater than those encountered ordinarily in the prepubertal child. During the first six weeks of therapy, excretion of estrogens was not altered appreciably, but thereafter no estrogenic activity could be detected in the urine, except sporadically. Assays revealed no gonadotropic activity except on two occasions, once before and once after therapy with cortisone was begun.

COMMENT

The administration of cortisone for thirteen months to a boy who ex-

hibited the characteristics of congenital adrenal hyperplasia produced no striking clinical changes. Perhaps some improvement in strength and cheerfulness occurred, and he continued to grow and mature physically. In the three years prior to treatment, he had grown approximately 4 inches (10 cm.) a year, whereas during the year in which cortisone was given, an increase of only $1\frac{3}{4}$ inches was noted. It is possible that this slowing of the rate of growth may have resulted from inhibition of the formation of androgens by cortisone, but an equally tenable explanation is found in the observation that the natural course of this disorder is characterized by cessation of growth and closure of epiphyses at about this age. This latter explanation is favored because of the further increase in bony age and beginning closure of epiphyses observed during therapy. It is possible that the use of somewhat larger doses of cortisone begun at an earlier age would have slowed epiphyseal closure, thereby permitting the boy ultimately to attain a greater stature.

The practice of Wilkins and associates (9) of using the excretion of 17-ketosteroids as a guide to dosage of cortisone was followed in this study, but our results do not permit evaluation of this principle, since no good clinical criterion was present by which to estimate response independently.

The rise in excretion of corticosteroids from somewhat low values when cortisone was administered is to be explained by the excretion of cortisone itself or of formaldehydogenic steroids that were derived from it (9). It has been suggested that excretion of small amounts of corticosteroids, reflecting a diminished production of cortisone-like steroids, may be characteristic of this syndrome (10).

Perhaps the most striking finding in this study was the nearly complete suppression of estrogenic activity of the urine. This is consistent with the concept that the adrenal in this syndrome is the source of estrogenic substances, and that cortisone is capable of preventing the formation of such substances, perhaps through inhibition of endogenous adrenocorticotropic hormonal formation. Another but less likely mechanism to explain the suppression of estrogenic activity can be postulated, however. This presumes that the administration of cortisone inhibits the release of a hormone of the pituitary which, in turn, is responsible for stimulating the formation of estrogen by peripheral tissues such as the testis.

Evidence has been presented to suggest that administration of cortisone to individuals who have congenital adrenal hyperplasia may release an inhibition of pituitary gonadotropic activity (9), but it is not surprising that definite gonadotropic activity did not appear in the urine of our patient when it is considered that his chronologic age was only 9 years.

SUMMARY

A 9-year-old boy who exhibited the characteristics of congenital adrenal hyperplasia was treated with cortisone for a year. Moderate increase in strength and endurance was noted. Decrease in the urinary excretion of 17-ketosteroids, increase in the excretion of corticosteroids and nearly complete suppression of estrogenic activity in the urine were noted as a result of this treatment.

Addendum

Since submission of this manuscript, a report has been published concerning the effect of cortisone on the excretion of fluorometrically measured estrogens by patients similar to the boy described in our report. Excretion of estrogens was found to be decreased but not abolished. (Migeon, C. J., and Gardner, L. I.: Urinary estrogens (measured fluorometrically and biologically) in hyperadrenocorticism: influence of cortisone, compound F, compound B and ACTH, *J. Clin. Endocrinol. & Metab.* **12**: 1513–1531 (Dec.) 1952).

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Letters to the Editor

ESTROGENIC ACTIVITY OF MONOBENZYL ETHER OF STILBESTROL IN THE HUMAN

To the Editor:

The monobenzyl ether of stilbestrol has been found to have a strong pituitary potentiating capacity in laboratory animals, but to exhibit a very weak estrogenic activity. According to the data in the literature, it has 1/10 to 1/5 the ovulation-inducing capacity of stilbestrol, but only 1/300 the estrogenic activity (1). Its effectiveness as a nonestrogenic stimulator of ovulation in humans has been reported (1).

This communication is to report a preliminary effort to establish its relative estrogenic effect on the human vagina, plans for a more complete definition of its human estrogenic activity having been interrupted by a call to active duty in the Armed Forces.

The method used for assay was that of Brown and Bradbury (2). Three groups of menopausal women were given priming doses of ethinyl estradiol, 0.05 mg. daily for ten days. When the effect on the vaginal smears had

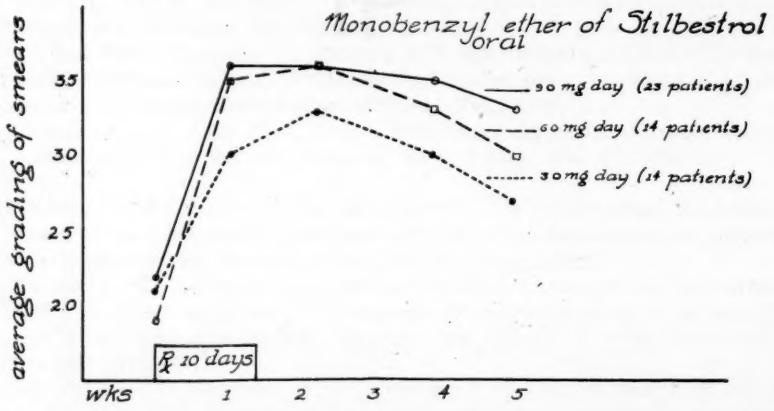

Fig. 1. Graphic representation of the average vaginal-smear response of the three groups of patients receiving monobenzyl ether of stilbestrol. Smears are graded by allotting a range of from 1.0 (atrophic) to 4.0 (completely stimulated). A response is considered to be positive if it rises above 3.0. From this diagram, it can be seen that the smallest dose of monobenzyl ether of stilbestrol (3.0 mg. daily for ten days) was sufficient to stimulate a positive vaginal-smear response.

worn off, each group received 3.0 mg. of monobenzyl ether of stilbestrol daily for ten days. The vaginal-smear response of each of these groups of women (14 to 23 patients per group) is presented graphically in Figure 1. For comparison, Figure 2 reproduces a similar assay of stilbestrol.

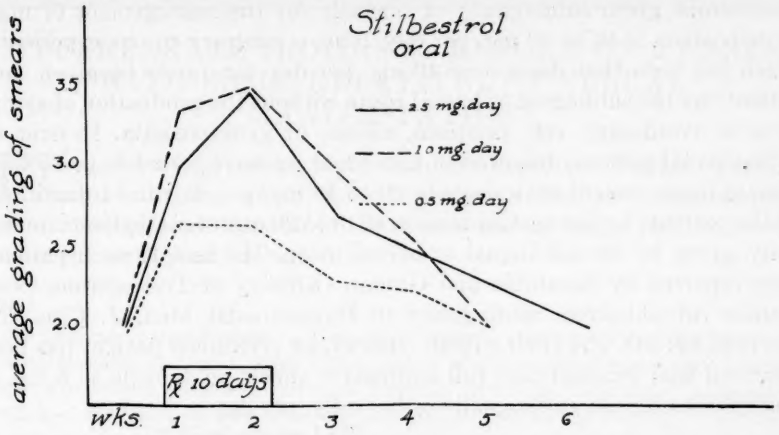

FIG. 2. Graphic representation of the assay curves for stilbestrol. This illustrates that 1.0 mg. of stilbestrol is the minimal dose which will give a positive response. (Reprinted from Brown, W. E., and Bradbury, J. T.: The use of the human vaginal smear in the assay of estrogens, *J. Clin. Endocrinol.* **9**: 725 (Aug.) 1949).

These data show that 3.0 mg. of monobenzyl ether of stilbestrol have at least the activity of 1.0 mg. of stilbestrol, so that in the human, monobenzyl ether of stilbestrol has at least $\frac{1}{3}$ the effect of stilbestrol on the menopausal vaginal smear. This is in contradistinction to the effect in the immature rat, in which the ratio is 1/300. Thus, the estrogenic activity of monobenzyl ether of stilbestrol should not be discounted in interpreting the clinical results obtained with this compound in the human female.

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DOSAGE OF METHYLTESTOSTERONE

To the Editor:

In the *Journal of Clinical Endocrinology and Metabolism* **13:** 235 (Feb.). 1953, Doctor Carl Heller is quoted as stating that the dose of methyltestosterone given sublingually or buccally for the management of male eunuchoidism is 40 to 80 mg. per day. This is contrary to our experience, which has been that doses over 40 mg. per day can rarely be given such patients by the sublingual or buccal route without the production of symptoms of overdosage, *viz.*, priapism, edema, or gynecomastia. In over 40 hypogonadal patients followed in this clinic we have found it possible to initiate improvement with doses of 20 to 40 mg. per day and to maintain all the patients by the administration of 10 to 20 mg. of methyltestosterone daily, given by the sublingual or buccal route. The first 12 such patients were reported by Escamilla and Gordan (Efficacy of Testosterone Compounds Administered Sublingually to Hypogonadal Men, *J. Clin. Endocrinol.* **10:** 248–264 (Feb.) 1950). Indeed, an occasional patient has been observed who demonstrates full androgenic effects on as little as 5 mg. of methyltestosterone, sublingually or buccally, per day.

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HYPERPARATHYROIDISM: REPORT OF 2 CASES WITH SOME RELATIVELY UNUSUAL MANIFESTATIONS

TWO cases of hyperparathyroidism are reported. Neither presents much in the way of difficulty in diagnosis, but each demonstrates one or more unusual features of the disease and problems that may arise during the postoperative period.

Case 1

The first case is that of **G.M.,** a 21-year-old Negress, who entered Highland Alameda County Hospital on April 5, 1952, with a variety of complaints of recent and of remote origin. For several years she had been generally unwell and had noted intermittent swelling of the hands, nausea, vomiting, nocturia, and occasional burning on urination. Some four years prior to entry, she first noticed vague pain in the left loin and occassionally in the left upper quadrant. Fever was alleged to have occurred with the onset of the pain. She had been subject to frequent headaches.

Examination disclosed a well nourished, and moderately well developed young adult Negress with dull apathetic facies, who responded vaguely to questioning. The temperature was 99° F., pulse 88, respiratory rate 26 and blood pressure 120/75 mm. of Hg. Pertinent physical findings included a mass of approximately 2 centimeters diameter in the region of the lower pole of the left thyroid lobe, "clubbing" of all fingers and both thumbs, and flattening, thinning and brittleness of the nails. In addition, there was some degree of hyperextension and shortening of the terminal phalanges. There was tenderness over the ribs, which proved to be the source of the pain described in the history. The lower extremities were normal, except for bilateral genu valgus.

Because of the urinary symptoms, a routine "flat plate" of the abdomen was ordered. The findings were interesting only from the standpoint of the visualized bony structures, and provided the first evidence of the underlying pathology. There was generalized demineralization of all of the visible skeleton, together with multiple cystic areas of rarefaction. Subsequently, the entire skeleton was examined by x-ray, and a similar pathologic condition was demonstrated throughout. A barium esophagram disclosed no deviation of the trachea. Dental films demonstrated absence of the lamina dura. Of particular interest were the films of the hands and feet. An increase in soft tissue density occurred about all of the terminal phalanges of both hands, in association with complete demineralization of the distal phalanges of both hands and feet, except for the proximal epiphyses. A presumptive diagnosis of osteitis fibrosa generalisata was made from the x-ray films. This diagnosis was confirmed by the finding of the following concentrations in the serum: calcium 21.2 mg. per 100 cc., phosphorus 2.0 mg. per 100 cc., and alkaline phosphatase 127 King-Armstrong units.

Immediately prior to surgery the patient was given a diet containing less than 200

milligrams of calcium per twenty-four hours (to minimize calcium excretion). Fluid intake was increased to 5 liters daily to maintain a dilute urine. Testosterone propionate and estrogen were administered to diminish bone catabolism.

On April 22, 1952, the patient was subjected to surgical exploration of the neck under general anesthesia. A mass measuring 2.0×2.5×1.5 centimeters was removed from the lower pole of the left thyroid by Dr. George Henegar. Grossly the mass was smooth, glistening, and mottled dark red and yellowish gray. Histologic examination confirmed the diagnosis of parathyroid adenoma.

For forty-eight hours postoperatively the patient was under continuous observation by teams composed of members of the house staff. An hourly record was kept of the urinary output and the urinary calcium, as determined by the Sulkowitch test. An adequate supply of calcium gluconate was kept at hand in the patient's room for emergency use. Blood samples were analyzed for calcium, phosphorus, sodium and potassium twice daily for the first seventy-two hours after surgery.

During surgery a marked diminution of the urinary output occurred. This persisted until the evening of the first postoperative day, when the output gradually increased to normal. The concentration of serum potassium, shortly after surgery, was 2.2 mEq./L. This decreased to 1.9 mEq./L. during the next twenty-four hours. During this period the patient had frequent emeses. A continuous intravenous drip of 5 per cent dextrose in distilled water with 1 gram of calcium gluconate per liter was maintained at a rate of 400 cc. per hour, until hydration was achieved. Potassium was added to the intravenous fluid after the output of urine began to increase.

The day following surgery the serum calcium level had dropped to 12.6 mg. per 100 cc. and the serum phosphorus to 1.2 mg. per 100 cc. Chvostek's sign made its first appearance on this day and continued throughout the period of hospitalization. The Trousseau test gave negative results throughout.

On April 25, 1952, a high-calcium, high-vitamin, soft diet was instituted, and two days later oral calcium lactate and potassium chloride were added to the regimen. Vitamin D, 7,500 units daily, was added on May 5, supplementing a standard multivitamin formula. By the fourth postoperative day, the patient was improving steadily and on the eleventh postoperative day, the serum calcium level was 9.2 mg. per 100 cc., serum sodium 136 mEq./L., and serum potassium 3.92 mEq./L. She was discharged from the hospital on the seventeenth postoperative day and was instructed to consume a high-calcium, high-protein, high-caloric diet, supplemented with calcium lactate and vitamin D.

The next chapter in her case history was obtained partially second hand. Immediately after release from the hospital, the patient left this area and on April 20, 1952, was admitted to another institution, in a state of tetany. The serum calcium and phosphorus levels were reported as 4.0 mg. and 2.7 mg. per 100 cc., respectively. She was treated with intravenous calcium gluconate, followed by 50,000 units of vitamin D orally three times daily, supplemented a few days later by dihydrotachysterol. The serum calcium level remained low in spite of therapy and on June 15, 1952, she manifested acute psychotic symptoms requiring commitment to a state hospital four days later. At this time she was confused, noisy, incoherent and required restraints and hydrotherapy. Treatment with supplemental calcium and vitamin D was continued, and on July 5, 1952, her serum calcium level was reported to be 14.45 mg. per 100 cc. and the serum phosphorus, 3.6 mg. per 100 cc. All medications were discontinued and on or about July 7, 1952, her behavior pattern became normal. However, by September 5, 1952, her serum calcium concentration had again fallen to 6.1 mg. and the serum phosphorus to

1.6 mg. per 100 cc. Therapy was re-instituted. Her condition continued to improve and she was finally discharged to her home.[1] She has been seen in our clinic several times since discharge from the State Hospital, and objectively her status, both physical and mental, is excellent. The serum calcium and serum phosphorus levels on October 10, 1952, were 7.6 mg. and 3.1 mg. per 100 cc., respectively. X-ray examination at this time showed essentially complete recalcification of the terminal phalanges of the hands. The remainder of the skeleton revealed less impressive recalcification.

Case 2

The second case is that of **M.E.,** a 57-year-old Negress, who was admitted to Highland-Alameda County Hospital on June 8, 1952. On the day of admission she had fallen and had injured her left hip. However, the history disclosed poor health since November 1951, at which time she first noticed easy fatigue, general malaise, and episodes of vomiting. Her appetite was poor and she had lost some 20 pounds of weight. Review of the systems was essentially negative, except for nocturia once nightly without burning. In 1946, she had undergone surgery for a fibroid tumor.

Examination disclosed a small (4 ft. 3 in.) thin Negress with a prominent kyphoscoliosis. The temperature was 99.2 F., pulse 96, respiratory rate 16, and blood pressure 120 /84 mm. of Hg. "Clubbing" of the fingers, especially on the right, similar to that in the case of G.M., was noted. There was tenderness over the left hip. A nodule in the left lower thyroid lobe was palpated with difficulty. A routine x-ray examination of the left hip and pelvis disclosed an intracapsular fracture of the neck of the left femur. In addition, multiple diffuse patchy areas of rarefaction of the bones of the pelvis and both femurs were noted by the roentgenologist. Because of the x-ray evidence of diffuse bone disease, films of the chest, spine, skull and hands were made. Anterior wedging and compression of the mid-dorsal vertebral bodies and diffuse demineralization of all visualized bony structures were observed. Evidence of an old healed fracture of the right clavicle was present. The bones of the skull, face, mandibles and cervical spine had undergone extensive demineralization. There was marked blunting and clubbing of the distal ends of all of the digits of both hands, with fraying of the distal tufts. A barium esophagram was reported as showing no deviation of the esophagus.

On July 16, 1952, a Smith-Peterson nail was installed in the left hip and a specimen of bone was removed for pathologic evaluation. This fragment of bone showed a picture characteristic of osteitis fibrosa generalisata, with narrowing and thinning of bony trabeculae, increased fibrosis of the marrow spaces, and increased osteoclastic activity. Laboratory studies disclosed a serum calcium level of 14.5 mg. per 100 cc., serum phosphorus 3.8 mg. per 100 cc., and alkaline phosphatase 81 King-Armstrong units; the blood urea nitrogen level was 42 mg. per 100 cc. The hemoglobin was 8.6 grams per 100 cc. Phenolsulfonphthalein excretion was 8 per cent in two hours.

Prior to parathyroidectomy the patient was started on a regimen similar to that of G.M., including testosterone propionate and estrogen. Whole blood transfusions were administered because of the low hemoglobin.

On July 22, 1952, surgical exploration of the neck was undertaken and a parathyroid tumor was removed by Dr. Arthur Hunnicutt from the left lower pole of the thyroid gland. The tumor appeared to be bilobular. One lobe measured $3 \times 1.5 \times 0.4$ centimeters

[1] Grateful acknowledgment is made to Dr. Walter Rappaport, Agnew State Hospital, and Dr. David L. Ichelson, Tulare County General Hospital, for information concerning the treatment and progress of Patient GM, while under their care.

and the other 2.2×1.5×0.4 centimeters. The weight was 4.5 grams. One hour following removal of the adenoma, carpopedal spasm developed; this was relieved by intravenous calcium gluconate.

Postoperatively, the patient was observed closely and the urine was tested hourly for calcium. Twenty-four hours after surgery the serum calcium and phosphorus levels were 11.2 mg. and 2.0 mg. per 100 cc., respectively. Her course was uneventful until 2:00 a.m. on July 27, 1952, when abdominal cramping developed and grossly bloody urine was passed a little later. The abdominal pain radiated to the left thigh. There was marked spasm of the left abdominal musculature with marked left costovertebral angle tenderness. X-ray examination of the abdomen failed to disclose any definite evidence of urinary tract calculus. The cramping and hematuria cleared rapidly. On the following day, the concentration of blood urea nitrogen had increased to 70 milligrams per 100 cc. and urine examination disclosed a trace of albumin, and 35 to 50 white blood cells per high dry field. The findings with the phenolsulfonphthalein test had improved, however, 20 per cent of the dye being excreted in two hours. E. coli was subsequently cultured from the urine. On July 30, 1952, a well marked Chvostek's sign was noted, which gradually cleared after intensive intravenous calcium gluconate therapy. At this time ingestion of milk and calcium lactate was begun, in addition to the intravenous medication. On August 2, 1952, vitamin D in a dosage of 10,000 units per day was added to the regimen. She was discharged on August 27, 1952. At the time of discharge, the fracture of the left hip was not united, but the patient was feeling well. Two months after removal of the parathyroid adenoma, she had gained 4 pounds and had no complaints other than those referable to her hip. On October 10, 1952, the serum calcium level was 7.6 mg. and the serum phosphorus, 3.7 mg. per 100 cc. A slight Chvostek sign was still present when the patient was last seen.

DISCUSSION

The 2 cases presented here bring out several points of general interest. The steady and unremitting character of the disease process in hyperparathyroidism may be so insidious and prolonged that generalized skeletal erosion develops before the underlying pathologic condition is recognized. Both patients had several features in common. Both were Negresses with widespread skeletal involvement, but without demonstrable renal calculi. In each, routine x-ray examination led to the diagnosis. Each had a nodule in the left lower lobe of the thyroid, one nodule easily palpable, the other felt with difficulty. Both had vague gastro-intestinal and genito-urinary complaints. These were the symptoms which resulted in hospitalization of one of the patients. The other was admitted for a hip injury that proved to be a pathologic fracture. Each exhibited clubbing of all of the digits of both hands, a relatively rare finding in hyperparathyroidism and probably referable to fractures of the terminal phalanges with telescoping of the soft tissues (1). This abnormality was equally distributed in both hands of one patient and was more prominent in the right hand of the other (Fig. 1).

In each patient the relatively unusual finding of a palpable mass in the neck was helpful in guiding the surgical procedure.

The postoperative difficulties encountered in Patient G.M. may occur

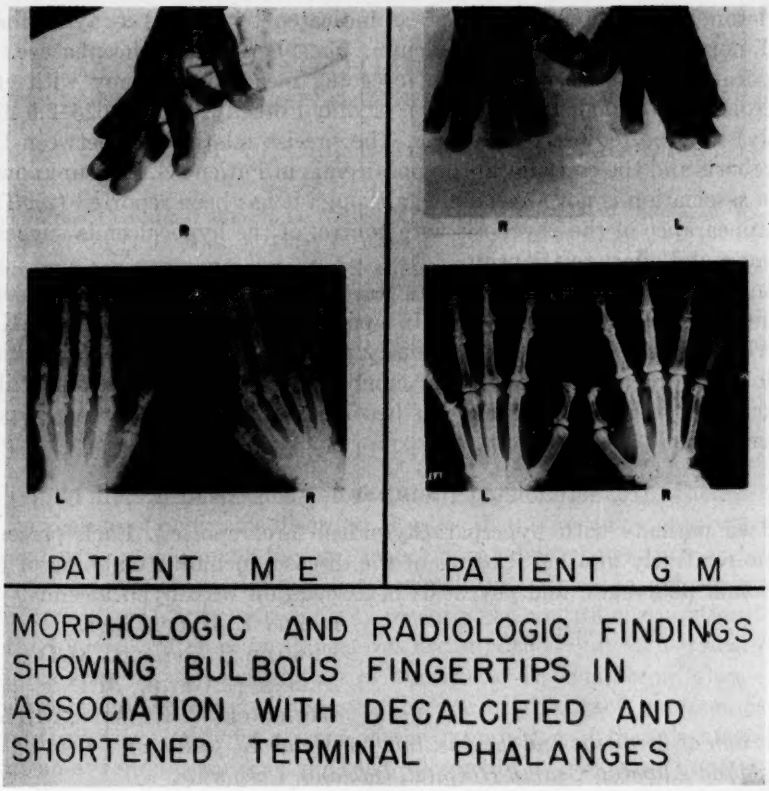
PATIENT M E
PATIENT G M
MORPHOLOGIC AND RADIOLOGIC FINDINGS SHOWING BULBOUS FINGERTIPS IN ASSOCIATION WITH DECALCIFIED AND SHORTENED TERMINAL PHALANGES

FIGURE 1

in any patient with extensive skeletal decalcification. The hypocalcemia is referable to the "hunger" of the decalcified skeleton and possibly to temporary hypofunction of the remaining parathyroids. It is, therefore, essential that in the immediate postoperative period a medical team be constantly on duty.

Procedures to be carried out include an hourly check of urine volume and urine calcium (with the Sulkowitch reagent), and infusion of calcium lactate, gluconate or chloride until tests show urinary calcium to be present. An indwelling catheter is desirable during the first forty-eight hours.

Adequate administration of fluid and of electrolytes must be attended to. The hypokaliemia in Patient G. M. was probably attributable to vomiting and dehydration.

After the initial postoperative phase, long-term therapy always calls for relatively high vitamin D, calcium and phosphorus intakes until the

skeleton is completely recalcified, as indicated by normal x-ray findings and normal blood levels for calcium, phosphorus and phosphatase. If hypocalcemia occurs despite the foregoing measures, therapy with calciferol (50,000–300,000 units daily)[2] or dihydrotachysterol (0.625–2.5 mg. daily) may be required for a time. The precise relationship between the psychosis and the concomitant hypocalcemia in Patient G.M. is unknown. The association is not a usual one, although it has been reported (2). The disappearance of the psychosis with control of the hypocalcemia suggests a cause-and-effect relationship.

In both patients Chvostek's sign was obtained in the absence of other clinical evidence of hypocalcemia. In a patient of one of the authors (L.K.), Chvostek's sign has been continuously present during a four-year period following surgery. Presumably its significance is that of a neuromuscular "excitability threshold" which has become conditioned to chronic hypercalcemia.

SUMMARY

Two patients with hyperparathyroidism are reported. Each presents some relatively unusual features of the disease, including clubbing of the terminal phalanges, and psychosis in association with hypocalcemia.

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[2] Grateful acknowledgment is made to Whittier Laboratories for the supply of Ertron used in the management of one of these patients (40,000 units = 1 mg. of calciferol).

Editorial

JOURNAL OF CLINICAL ENDOCRINOLOGY
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Managing Editor: Willard O. Thompson.

THE NEED FOR AN INHIBITOR OF GONADOTROPIN

IT IS of interest to speculate on the future possibilities of the therapeutic correction of endocrine disorders. Earlier the major advances were concerned with replacement therapy in hormonal deficiencies such as hypothyroidism, diabetes insipidus, diabetes mellitus, adrenal insufficiency and hypogonadism. Until comparatively recently the control of conditions due to the hypersecretion of hormones was accomplished only by surgical procedures such as the extirpation of tumors of the endocrine glands or thyroidectomy in Graves' disease. The use of thiouracil compounds in hyperthyroidism and of cortisone in the suppression of the adrenogenital syndrome were advances in the medical control of glandular hypersecretion. There is obvious need for similar measures in the treatment of other disorders due to endocrine hyperactivity. In particular it would be most desirable to find some method of suppressing the secretion of pituitary gonadotropins. This might lead to the prevention or control of sexual precocity of the "constitutional type," the correction of certain menstrual and menopausal disorders, the regulation of fertility and the suppression of some gonadal neoplasms.

There have been a number of different approaches to the problem of suppressing the secretion of pituitary gonadotropins or of antagonizing their effects upon the gonads:

It has been shown that gonadotropins prepared from the pituitary of one animal cause the formation of "antigonadotropins" when injected into an animal of a different species. This suggested the possibility that the prolonged administration of heterologous gonadotropins in humans might lead to the formation of antibodies against gonadotropin endogenously secreted by the pituitary, or that "antihormones" might be prepared for

therapeutic use. Such attempts seem unlikely to succeed, since most evidence indicates that the resistance produced by repeatedly injecting a particular gonadotropin is due largely to immunity developed against a foreign protein or protein-like body and cannot be described correctly as antigonadotropic (1, 2).

In 1941 it was announced that a brew made from a weed known as *Lithospermum ruderale* was imbibed by squaws of the Indian tribes of Nevada as a contraceptive to prevent the production of papooses. This led to extensive studies by Cranston (3, 4) of the Pharmacology Department of the University of Minnesota, Drasher (5) of Indiana University and Plunkett and Noble (6) at the University of Western Ontario. The substance seems to cause a prolonged diestrus in mice and rats by blocking the secretion of luteinizing hormone; but there are some toxic side effects and the proof of its action is not conclusive. Certainly the active principle is not now applicable to therapeutic use in humans.

In 1935 D. R. McCullagh and Walsh (7) postulated that the testes secreted a hormone other than androgen or estrogen, which they obtained in water extracts and termed "inhibin" because of its ability to inhibit the secretion of pituitary gonadotropin. Other workers have been unable to repeat these findings. Klinefelter, Reifenstein and Albright in 1942 (8) explained the gynecomastia and high FSH secretion in the syndrome of hyalinization of the seminiferous tubules on the basis of absence of an inhibitory "x-hormone" supposedly secreted by the Sertoli cells; and Howard, Sniffen, Simmons and Albright in 1950 (9) suggested that the "second hormone of the testes" might be Δ^5-pregnenolone. Most workers now believe that estrogen is secreted by the testis but there is difference of opinion as to whether it is produced by the Sertoli cell or the Leydig cell. At least no specific inhibitor of FSH, devoid of other action, has been demonstrated.

In 1949 a number of French investigators (10, 11) claimed that parahydroxypropiophenone, a synthetic chemical, blocked the secretion of both thyrotropin and gonadotropin. It was stated that the pituitary inhibition occurred in the absence of estrogenic action. These overenthusiastic claims have been refuted by a number of American workers. Heller and associates (12) showed that the drug failed to prevent the postcastration rise of gonadotropins in rats.

The possibility remains that some steroid, or other chemical structurally related to the sex hormones, may be found capable of inhibiting the secretion of pituitary gonadotropin while causing little or no estrogenic or androgenic manifestations. Mortimer, Paulsen and Heller (13) studied 14 steroids, including Δ^5-pregnenolone, which are relatively inert biologically but which are chemically similar to estrogen, androgen or progesterone;

they did not find one which inhibited the increased secretion of gonadotropin following castration in rats.

Although the search for a specific inhibitor of gonadotropin has been unproductive, there remains ample opportunity for research in this field.

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Questions and Answers

(Questions submitted at the 1952 Postgraduate Assembly of the Endocrine Society)

Answers by Paul Starr, Los Angeles, California

QUESTION: Is TSH available commercially? If not, where can it be obtained?

ANSWER: TSH is not available commercially. It may be obtained only for investigational use, from the larger companies producing pituitary hormone preparations, if their supplies are adequate.

QUESTION: Please comment on the incidence and interpretation of acute onset of Graves' disease following severe emotional trauma.

ANSWER: A common clinical story is the association of the onset of Graves' disease with severe emotional trauma. Whether or not the trauma is actually causative is not known, but it seems highly probable in some cases. It should be equally recognized that the subclinical state of hyperthyroidism predisposes to an exaggeration of the response to emotional trauma.

QUESTION: Please discuss the treatment of solitary nontoxic nodules of the thyroid.

ANSWER: Single (solitary) nodules of the thyroid which are definitely not associated with goitrous enlargement of the remainder of the thyroid gland, should be removed surgically and studied for the presence of malignancy. The absence of palpable enlargement of the non-nodular thyroid tissue is evidence that the single nodule palpated is a neoplasm, not necessarily malignant, but with a high probability of becoming so.

QUESTION: If there is a somatic hypothyroidism in which the circulating thyroid hormone is "blocked from action," how can the circulating PBI determination be of help?

ANSWER: The syndromes at present identified as somatic hypothyroidism, *e.g.*, nephrosis, are associated with subnormal or low serum protein-bound iodine values.

QUESTION: Is the administration of thyroid hormone a good therapeutic test for hypothyroidism? What dose of desiccated thyroid daily would be considered enough to make this test?

ANSWER: The administration of thyroid hormone is not a good therapeutic test for hypothyroidism. The diagnosis should be made with the aid of the serum protein-bound iodine measurement. In the absence of this test, however, objective changes in the patient during thyroid therapy, such as an advance in bone age in children or improvement in ovarian cycles in women, may be used as a therapeutic test. Dosage of the order of 2 grains of desiccated thyroid daily should be sufficient.

Answers by Rulon W. Rawson, New York, N. Y.

QUESTION: Please discuss the use of x-ray to the pituitary in treatment of exophthalmos.

ANSWER: X-ray of the pituitary has been reported to have a beneficial effect in certain patients with severe exophthalmos. The dose of irradiation administered is not great enough to alter the function of normal pituitary tissue. It is possible that the beneficial effect described is due to a certain amount of irradiation which strikes the retro-orbital tissues. The writer has seen beneficial effects from small doses (less than 1,000 r) of x-radiation directed at the retro-orbital tissues in some of these patients.

QUESTION: Contrast medical treatment alone, with surgical treatment in exophthalmic goiter.

ANSWER: Under the entirely medical treatment of exophthalmic goiter, we have several modalities. In a small percentage of patients, prolonged treatment with iodine results in control of the disease. In a majority of patients treated for one year or more with any of the thiouracil derivatives, adequate control of the disease is obtained. Unfortunately, only about 50 per cent of those controlled with these antithyroid drugs remain under control after stopping the drug. The best results are obtained in the younger women; the poorest results are obtained in males or in people with very large goiters. Radioactive iodine is likewise a very effective, though destructive, method of controlling the hyperthyroidism of Graves' disease. This treatment in most clinics is limited to patients of the older age group. Patients adequately treated with radioactive iodine rarely, if ever, experience recurrences. Surgical treatment of Graves' disease after adequate preparation with one of the antithyroid drugs and iodine is a very satisfactory way of controlling the hyperthyroidism. There are, however, the risks of nerve damage or of parathyroid tetany, and the recurrence rate varies between 5 and 10 per cent.

QUESTION: Discuss treatment of hyperthyroidism with radioactive iodine alone. Is it reliable?

ANSWER: Hyperthyroidism has been treated successfully with radioactive iodine in a significantly large series of cases in several clinics. It appears to be one of the most reliable forms of treatment available, if adequate doses of radioiodine are administered. Determination of an adequate dose depends upon accurate estimation of the size of the thyroid gland and upon measurement with tracer techniques of the amount of radioiodine retained by the thyroid. The dose which the writer tries to deliver in treating such patients is 140 microcuries per estimated gram of thyroid tissue. Results of this dose in patients having diffuse thyroid hyperplasia are usually excellent. There are instances, however, in which repeated treatments are necessary. This need for additional therapy is not determined until at least three months have elapsed following the previous treatment. The treatment with radioactive iodine of hyperthyroidism arising from hyperfunctioning adenomas of the thyroid requires larger doses of radioactivity than those used in classic Graves' disease.

QUESTION: Comment on the treatment of exophthalmos that remains after operation.

ANSWER: The treatment of severe exophthalmos that remains or develops after operation presents one of the most difficult therapeutic problems faced by the physician. Some patients are benefited by the administration of thyroid in doses necessary to cause a

slight elevation above normal in the BMR. X-radiation of the orbit has been observed to be beneficial in patients whose exophthalmos threatens their eyes. Treatment with diuretic agents has been observed to relieve some of the tension behind the eyes in patients with severe exophthalmos. The most beneficial trick used by the writer is that of having the patient sleep with the head elevated. This results in less storage of water behind the eyes.

Answers by Peter H. Forsham, San Francisco, California

QUESTION: What is the incidence of Addison's disease in the over-all population of the U.S.?

ANSWER: Based on older postmortem findings, the incidence of Addison's disease in its full blown clinical form does not exceed 1 in 30,000. Clinically the figure may lie between 1 in 10,000 and 1 in 20,000. It is thus a rare disease.

QUESTION: Has cortisone decreased the incidence of sudden death in Addison's disease?

ANSWER: The incidence of sudden death in Addison's disease was rare and essentially unexplained in the past. It was, however, thought to be due most often to either a very high serum potassium concentration (in the untreated patient) or a very low one (in patients over-treated with desoxycorticosterone). With the advent of desoxycorticosterone as a therapeutic agent and its judicious use, sudden deaths no longer occur. Although cortisone has added immeasurably to the attainment of normalcy in terms of weight, hemoglobin, feeling of well being, and strength in patients with Addison's disease, it came too late to influence the rare incidence of sudden death.

QUESTION: Would determination of 17-ketosteroids during pregnancy give an indication of the desoxycorticosterone level or the level of adrenal cortical activity?

ANSWER: Determination of 17-ketosteroids during pregnancy would not yield any information as to the level of desoxycorticosterone-like substance in the body, since the end product of this substance is not 17-ketosteroid but rather pregnandiol. As a measure of adrenal cortical activity it is none too valuable, since 17-ketosteroids are formed in large measure in the liver and the function of this organ may be disturbed. Furthermore, using the Zimmermann reaction for the determination of 17-ketosteroids, high values are obtained in pregnancy because 20-CO compounds related to progesterone also give the color. This disadvantage may be overcome by using the antimony trichloride method of Pincus.

QUESTION: How long after the menopause does FSH or gonadotropin excretion remain high? Is there evidence (vaginal smear, etc.) of decreasing estrogen deficiency as the symptoms of the menopause subside?

ANSWER: FSH or gonadotropin excretion usually remains elevated for from five to ten years after the onset of the menopause and not infrequently up to the time of death. There is no evidence for decreasing estrogen deficiency after the symptoms of the menopause subside and such an effect is usually attributed to an adaptation of the body to the vegetative disturbance which goes with the menopause and of which FSH is only an indirect indicator.

Answer by Norris J. Heckel, Chicago, Illinois

Question: Have you any explanation for the continued excretion of 17-ketosteroids in the urine of males with prostatic carcinoma following bilateral orchiectomy and adrenalectomy? Where do these 17-ketosteroids come from?

ANSWER: It is not definitely known where the 17-ketosteroids come from. One possible explanation is that they come from misplaced adrenal tissue. Observations from autopsies have shown that adrenal tissue occurs not uncommonly in parts of the body other than the usual location of the adrenal glands.

Answers by Willard M. Allen, St. Louis, Mo.

QUESTION: What is the value of a smear of breast secretion in the diagnosis of breast cancer?

ANSWER: I have no experience with the use of the smear of breast secretion in the diagnosis of breast cancer. This method is used very little by our surgeons. They rely almost entirely on biopsy and an immediate study of a frozen section. If the section gives positive findings, a radical mastectomy is performed without delay. If there is doubt regarding a definite diagnosis from the frozen section, the mastectomy is postponed until fixed and stained sections can be studied.

QUESTION: How do you explain and treat persistent colostrum secretion in single women?

ANSWER: Persistent colostrum secretion in single women who have regular periods and no evidence of endocrine dysfunction usually requires no treatment. The most important advice to give the patient is to insist that she not manipulate the breasts or nipples to see if there is colostrum.

QUESTION: What percentage of artificial inseminations result in pregnancy?

ANSWER: When good donors are used for artificial insemination and the woman has normally functioning reproductive organs, pregnancy has occurred in 80 per cent of the patients inseminated monthly over a period of from four to six months.

When the husband's semen has shown a low count and has been used for artificial insemination, pregnancy has resulted in about 1/3 of the cases.

Answers by Roberto F. Escamilla, San Francisco, California

QUESTION: How does methyltestosterone compare with free testosterone or testosterone propionate when used for protein anabolism?

ANSWER: Methyltestosterone is as effective as testosterone propionate and superior to free testosterone when used for protein anabolism. From the standpoint of convenience and economy it is advantageous to use methyltestosterone, which can be given orally or sublingually, rather than the other preparations, which must be administered parenterally to be effective.

QUESTION: Does methyltestosterone cause jaundice, and what effect has it on the liver?

ANSWER: No causal relationship has been established between methyltestosterone therapy and jaundice. A few cases of jaundice occurring during methyltestosterone therapy

have been reported, but considering the many thousands of patients to whom the drug has been administered, the incidence is extremely low and probably not above that to be expected from viral hepatitis; moreover, the jaundice will clear while this medication is continued. Recently it has been reported that methyltestosterone is beneficial in relieving the pruritis of jaundice (*Brit. M. J.* **2**: 1289 (Dec. 13) 1952). The effect on the liver has been studied by Werner and associates, who report that a type of bile stasis results from its use. Because of this finding, it is probably safest not to use methyltestosterone in a patient with proven liver disease.

QUESTION: Why is there no elevation of the plasma cholesterol level in pituitary myxedema?

ANSWER: Reasons for the normal plasma cholesterol level in pituitary myxedema are not clear. Furthermore, it is not always normal but may occasionally be elevated to as high as 500 or 600 mg. per 100 cc. A suggested explanation is that the removal mechanism for cholesterol, which is interfered with in primary thyroid myxedema, is normal in pituitary myxedema, and that therefore the level of plasma cholesterol reflects the amount of fat taken in the diet. A recent report that the plasma cholesterol concentration tends to be low in adrenal cortical insufficiency suggests this as another possible factor. Perhaps the myxedema seen in hypopituitarism is not due to secondary hypothyroidism; this is further suggested by the fact that these patients usually do not react well to treatment with desiccated thyroid and occasionally acute adrenal cortical insufficiency may be precipitated by its use.

However, since the plasma cholesterol level is usually normal in pituitary myxedema, the combination of myxedematous appearance and normal plasma cholesterol level should suggest primary pituitary disease.

QUESTION: Please explain methods to differentiate "pituitary" Addison's disease from "true" Addison's disease.

ANSWER: 1. The *clinical history* may be helpful. Early loss of sex function and other signs of hypopituitarism, including unexplained loss of weight, may have been present before the typical signs of adrenal cortical insufficiency, and should suggest the possibility of primary pituitary insufficiency.

2. *X-ray examination of the sella turcica* may reveal the presence of a nonfunctioning pituitary tumor, such as chromophobe adenoma or craniopharyngioma. If found, either would indicate a primary hypopituitarism.

3. *X-ray examination of the adrenal regions.* Since a fair number of cases of Addison's disease are still caused by tuberculosis, the finding of calcification in the adrenal regions suggests this etiology, and therefore "true" Addison's disease. The finding of signs of tuberculosis in a chest roentgenogram would also be a point in favor of primary adrenal disease.

4. Response of the *circulating eosinophils* to ACTH stimulation may be helpful. Both the 4-hour intramuscular test and the 8-hour intravenous test can be used, although the latter usually requires hospitalization for the infusion. If the eosinophils drop 50 per cent or more, the adrenals would appear capable of function with proper stimulation, indicating primary hypopituitarism. However, this is not an invariable result.

Otherwise the clinical picture of the two conditions may be similar and differentiation difficult. If the distinction can be made, some of the patients with "pituitary" Addison's disease will do better on treatment with ACTH than with cortisone and/or desoxycorticosterone acetate.

QUESTION: Is it not true that a negative result from the Sulkowitch test (showing absence of calcium in the urine) is of more value than a positive result?

ANSWER: No. Both a negative result from the Sulkowitch test (clear) and a strongly positive result (4 plus—solid cloud) are of equal and considerable importance when run on a urine specimen from the fasting patient:

A negative result indicates any of the following:

1. Blood serum calcium level of 7.5 mg. per 100 cc. or under, in the presence of a normal serum total protein level—as in hypoparathyroidism (tetany),
2. Decreased absorption of calcium—as in steatorrhea,
3. Increased uptake of calcium by bone,
4. Decreased rate of breakdown of bone.

A strongly positive result may indicate:

1. Hypercalcemia—blood calcium level over 11 mg. per 100 cc.—as in hyperparathyroidism,
2. Increased absorption of calcium—as in vitamin D intoxication,
3. Increased breakdown of bone—as in Paget's disease of bone or in severe osteoporosis.

The positive result has more value if the degree is reported, *i.e.*, 1 plus (slight cloud), 2 plus (moderate cloud), 3 plus (heavy cloud), or 4 plus (solid cloud). The rapidity of development of the cloud is also helpful in indicating the amount of calcium present in the urine. Normally the fasting urine specimen shows a 1 to 3 plus reaction.

If a strongly positive reaction is found, the patient can be given a low calcium diet, excluding particularly milk and cheese, and any calcium-containing medications; after three or four days the Sulkowitch test can be repeated on a 24-hour urine specimen. If the reaction is still 3 or 4 plus, it is more strongly indicative of the conditions listed.

If the Sulkowitch test is made part of the routine urine examination, the finding of a 4 plus reaction may bring to light a hitherto undiagnosed or unsuspected case of hyperparathyroidism.

QUESTION: Which glucose tolerance test gives the most information in endocrine problems?

ANSWER: Although criticized at times, the standard method of performing the glucose tolerance test remains, for practical purposes, quite satisfactory for the diagnosis of endocrine problems. This method uses the ingestion of 100 grams of glucose (less in children). For the determination of glucose concentration, blood and urine samples are taken fasting, and at one-half, one, two and three hours, and occasionally four, five and six hours after administration of the glucose.

For the diagnosis of diabetes mellitus, or to reveal a diabetic tendency in other diseases such as Cushing's syndrome or acromegaly, a 2-hour or 3-hour test is satisfactory.

For the diagnosis of hypoglycemia, the test should be run for five or six hours, as the hypoglycemic tendency may not appear until after four hours. Should the patient have any symptoms suggesting hypoglycemia during the test, an extra blood specimen should be taken at that time, even though it is not on the regular hourly schedule. In this manner, a hypoglycemic dip in the curve may be discovered which otherwise would be missed. When hypoglycemia is due to a tumor of the pancreatic islet-cells, a low fasting level is the most important, and it may be necessary to have the patient fast from twenty-four to forty-eight hours to emphasize this tendency. Special dietary preparation usually is not necessary, for if the carbohydrate intake has been between 100 and 300 grams daily, the glucose tolerance test will give a satisfactory indication of the manner in which the patient handles glucose.

QUESTION: Would you recommend long-action insulin, such as protamine-zinc insulin, to increase the appetite?

ANSWER: No. Regular insulin should be used, as the desired effect is a sharp temporary drop of the blood sugar level with meals, so that the appetite will be stimulated only at that time. Protamine-zinc insulin, with its delayed action, would not give this result and might cause hypoglycemic reactions at unpredictable and inconvenient times.

QUESTION: Insulin has been suggested in the treatment of rheumatic fever. Why?

ANSWER: Insulin in combination with cortisone has been suggested in the treatment of rheumatic fever. The probable basis is the observation of Henderson *et al.* (*J. Clin. Endocrinol.* **10:** 800 (July) 1950) that insulin may act as a synergist to cortisone in the treatment of rheumatic disease, and that smaller amounts of cortisone may be effective when the combination is used. Suggested daily doses are 25 to 50 mg. intramuscularly of cortisone plus 40 units of regular insulin in single or divided doses.

The important consideration in rheumatic fever is whether cortisone is as beneficial as it was originally thought to be in the condition. This matter is now the subject of debate and further investigation. If cortisone is not used, there seems no indication for the use of insulin, except perhaps in a general way, if indicated as a stimulant of the appetite.

Answers by Frank L. Engel, Durham, N. C.

QUESTION: What is your opinion on invert-sugar carbohydrate metabolism and diabetes?

ANSWER: The demonstration by a number of investigators, using a variety of techniques, that fructose utilization is normal in the diabetic organism has evoked considerable interest in the use of this material, both in diabetes and for intravenous alimentation in other conditions. Fructose enters the metabolic pool as fructose-6-phosphate with the aid of the enzyme fructokinase. In contrast to the enzyme hexokinase which catalyzes the phosphorylation of glucose, fructokinase activity does not seem to be influenced by the hormones of the pancreas, adrenal cortex or anterior pituitary. Invert sugar is the product of hydrolysis of sucrose, *i.e.*, glucose and fructose. The use of either invert sugar or of fructose alone intravenously makes carbohydrate available for the diabetic and the postoperative or ill patient in whom glucose is ordinarily uneconomically utilized. It is altogether likely that in the treatment of diabetic acidosis, the early use of fructose with insulin may more rapidly overcome ketosis without the dangers of increased cellular dehydration which results from the increased hyperglycemia associated with the early use of glucose in the treatment of diabetic coma. Furthermore, it should effectively prevent the hypoglycemia which occasionally occurs when insulin and saline alone are used. It is unlikely that invert sugar or fructose by mouth will have any useful role in the management of diabetes.

QUESTION: Please comment on the metabolic basis of two current practices. 1) Kinsell advises high-fat diet during treatment by ACTH or cortisone, whereas Seneca advises insulin during such treatment. Does either seem rational? 2) Pennington advises reducing overweight persons by high-fat, high-protein, carbohydrate-free, low-calorie diets.

ANSWER: 1) Kinsell has reported that extremely high fat diets may prevent some of the untoward effects of ACTH or cortisone treatment without interfering with the therapeutic effects. So far, corroboration of this observation has not yet been reported, but

Kinsell himself concedes that this is neither a practical nor advisable dietary regimen for such patients (*Ann. Int. Med.* **37**: 921, 1952). Most investigators are in agreement that, in general, the sodium intake should be restricted, preferably to 200 mg. or less and the potassium intake increased. For the latter purpose, 8 ml. t.i.d. of an aqueous mixture ("KABC") containing potassium acetate 1 Gm., potassium bicarbonate 1 Gm., and potassium citrate 1 Gm., mixed with fruit or tomato juice, has proven satisfactory in this clinic. There is no complete agreement concerning the most desirable composition of the diet with respect to protein, carbohydrate and fat, but in all likelihood a diet comparatively high in protein and fat and low in carbohydrate is desirable. Total calories should be adjusted so as to allow a gain to, or maintenance of, optimal weight by thepatient. Undue weight gain should be avoided.

The combined use of insulin and cortisone has been recommended as a technique for increasing the efficacy of cortisone (*J. Clin. Endocrinol.* **11**: 119, 1951). Although this suggestion is well worth further investigation, the evidence that this combination is significantly more efficacious than the same dose of cortisone alone is not yet adequate, and hence it cannot be recommended at present as a routine method of treatment.

2) Given the same degree of caloric restriction which is the keystone of all reducing regimens, a high-protein, high-fat, carbohydrate-free diet as recommended by Pennington has several potential advantages over more conventional diets containing carbohydrate. First, such a diet has a higher satiety value than one containing more carbohydrate and hence is apt to be more satisfying. Second, restriction of carbohydrate in the diet results in less fluctuation of the blood sugar level, with a tendency to its stabilization at a somewhat higher level during fasting. According to Mayer and Bates' glucostatic theory of appetite (*Am. J. Physiol.* **168**: 812, 1952), this should result in less stimulation of appetite than if the blood sugar fell to lower levels. Finally, some advantage may accrue from the depression of lipogenesis known to occur when carbohydrate is eliminated from the diet.

Answers by Francis D. W. Lukens, Philadelphia, Pa.

QUESTION: Do you think that the frequent appearance of diabetes at or near the menopause is a manifestation of overstimulation or secretion of anterior pituitary hormones and is sometimes improved by the use of estrogen?

ANSWER: Although there is reasonably good evidence for an increased gonadotropic and adrenotropic secretion at the menopause, these changes do not cause diabetes in the majority of people. At this age the patient, but rarely the diabetes, may be improved by estrogen treatment. On the average, twenty years of obesity precedes the occurrence of diabetes in menopausal women and this seems to be a far greater evil than the physiologic changes in the hormones.

QUESTION: Does the administration of insulin to a diabetic depress the formation of insulin by the pancreas in the same way that the administration of other glandular substances depresses the respective glands?

ANSWER: The administration of insulin to a normal animal depresses the formation of insulin (Haist, *et al.*). In man, this may be illustrated by the transitory glycosuria which follows the removal of an island-cell adenoma. Whether the secretion of insulin is suppressed by the high level of blood insulin, the low level of blood glucose or other factors, is not settled, but the level of the blood glucose is certainly important. However, in the diabetic patient no such depression of insulin formation is demonstrable, perhaps be-

cause the ineffective islands are working at top speed or perhaps because the intermittent effect of administered insulin falls short of the natural requirements so that the islands are not continually "rested." In practice, "rest" of the islands of the diabetic by means of insulin appears to be one of the factors responsible for the improvement which is occasionally observed in patients treated early and well.

QUESTION: What is your opinion concerning the length of time a "new" diabetic is given to control hyperglycemia on diet alone?

ANSWER: A "new" diabetic, *i.e.*, one seen at the time of first diagnosis, is treated by diet alone (a) if adult and obese, or (b) if adult and more than 65 years old, even if thin, because diabetes in each of these groups is often mild. In children or thin diabetics of younger age, insulin should be started at once in most instances, because the disease is usually severe. The effect of dietary restriction seems to be twofold. First, there is the improvement which promptly follows the reduction in the daily food intake and which is seen within a week. Second, there is a slower improvement due to progressive weight loss. It may take months to demonstrate this. Usually it seems safer to work for this late effect under the protection of a small dose of insulin. The need for relieving symptoms of diabetes promptly, the presence of any complication, the cooperation of the patient and other factors will influence the physician's judgment. However, any diabetic not controlled on a 1300-calorie diet in a week forces one to think of insulin.

QUESTION: Please discuss various types of glucose tolerance curves considered diagnostic of diabetes mellitus.

ANSWER: The oral glucose tolerance curve considered characteristic of diabetes will have one or more of the following characteristics: 1) "Undue prolongation of hyperglycemia has been recommended as the best single criterion for the diagnosis of diabetes. A venous blood sugar greater than 120 mg. per cent three hours after a 100-gram dose of glucose is strong, if not conclusive, evidence of diabetes. After a dose of 50 grams an equal hyperglycemia after two and a half hours has the same significance. . . . While it is not desirable to depend in doubtful cases upon a single criterion, prolongation of hyperglycemia is more definite proof that glucose utilization is retarded than is an exceptionally high peak." (Peters and Van Slyke: Quantitative Clinical Chemistry, ed. 2, vol. 1, p. 305, 1946). 2) A fasting blood sugar above 110 mg. per 100 cc. (Somogyi method). If the morning blood sugar is grossly elevated (*e.g.*, 150 mg. per 100 cc. or higher) confirmation of this fact is diagnostic and a tolerance test is unnecessary. In view of the many irregularities of the sugar tolerance test, some of the curves obtained will not satisfy the usual criteria of normal or abnormal. The condition of the patient, the dietary preparation for the test, the physician's judgment and, frequently, the repetition of the test or the occasional determination of blood sugar concentrations for a period of months, must all be employed in making the diagnosis.

BOOKS RECEIVED

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